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PROCEEDINGS

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CONTENTS.

SCIENTIFIC PROCEEDINGS (50th–54th meetings):

PAGE

Communications of the fiftieth meeting, October 16, 1912 . . . 1
Communications of the fifty-first meeting, December 18, 1912 . . 23
Communications of the fifty-second meeting, February 19, 1913 . 65
Communications of the fifty-third meeting, April 16, 1913. . . 123
Communications of the fifty-fourth meeting, May 21, 1913 . . 155
Recapitulation of the names of the authors and of the titles of the communications 193
EXECUTIVE PROCEEDINGS (50th–54th meetings) 203
REGISTER OF NAMES AND ADDRESSES OF THE MEMBERS . . . 207
LIST OF OFFICERS 215
CLASSIFIED LIST OF MEMBERS. 217
INDEX OF THE SCIENTIFIC PROCEEDINGS. 221

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SCIENTIFIC PROCEEDINGS.

Abstracts of the Communications.

Fiftieth meeting.

Cornell University Medical College. October 16, 1912. Dr. Lee in the chair.

1 (697)

A note on the mode of infection in epidemic poliomyelitis.

By **Simon Flexner** and **Paul F. Clark.**

[*From the Laboratories of The Rockefeller Institute for Medical Research.*]

Both in experimental and human epidemic poliomyelitis the virus has been repeatedly demonstrated in the tonsils, in the nasal mucous membrane, and in nasal washings, both from fatal and acute cases. As the experimental disease can also be produced by intranasal swabbing with the active virus it seems probable that the nasal mucosa is one at least of the sources of the virus in the outside world and also the means of its entrance to the body. The marked viability of the virus under adverse conditions such as drying, low temperature, etc., must also be considered as making for a fairly well founded theory of the nasal route as one path of the virus to and from the body.

The precise manner in which microörganisms enter the body through mucous membranes is difficult to establish. Because we can produce experimental poliomyelitis by the application of the active virus to the nasal mucous membrane, we have in this disease a means of determining whether the virus so applied first enters the blood stream and through this the central nervous system or whether it ascends directly along the lymphatics that unite the nasal mucosa with the central meninges. In experimental poliomyelitis produced by any method of injection it is well known that the virus is present throughout the central nervous system. But after an intranasal injection, can the virus be demonstrated equally early in all regions of the cord?

In order to answer this question, the nasal mucous membrane of a *Macacus rhesus* monkey was swabbed lightly with a portion of ground cord from a recently paralyzed monkey. The monkey was killed at the end of 48 hours and the following portions of the central nervous system were removed separately and aseptically: (1) the olfactory lobes with small portions of the adjacent brain substance, (2) the medulla, and (3) pieces of the cord at different levels including the cervical and lumbar enlargements. These different portions were injected separately as suspensions into the brain and peritoneal cavity of three other *Macacus rhesus* monkeys.

The monkey injected with the suspension of the olfactory lobes came down in a manner typical of poliomyelitis in monkeys with definite prodromal symptoms on the ninth day, paralysis on the tenth and death on the twelfth day. At autopsy, lesions characteristic of the disease were observed throughout the cord.

The two other monkeys remained entirely well and have never shown any symptoms of paralysis.

The result of this experiment is definite. The virus of poliomyelitis passes from the nasal mucous membrane to the olfactory lobes and adjacent parts of the brain before it reaches the medulla or cord. This distribution is what we would expect were the ascent by the direct lymphatic path and not by the blood stream. Were the dissemination by the latter route we should expect early localization in those parts of the cord and medulla that possess an especial affinity for the virus.

2 (698)

Effects on meningeal tuberculosis of the local injection of foreign leucocytes.

By WILFRED H. MANWARING.

[*From the Rockefeller Institute for Medical Research.*]

In a previous report,[1] it was shown that the use of foreign leucocytes as a local therapeutic agent in experimental meningeal infections in dogs is limited by the toxicity of foreign leucocytes

[1] Vol. IX, p. 117.

for these animals. Rabbit leucocytes injected into the meningeal cavities of dogs invariably cause death. A single injection of horse leucocytes, however, can be safely made in normal dogs, although on repeating the injection, or on injecting horse leucocytes for the first time into meninges already the seat of an inflammatory lesion, death results.

The injection of horse leucocytes into the cerebral meninges of dogs, simultaneously with the inoculation of the meninges with tubercle bacilli, causes a slight delay in the development of the paralytic symptoms in about half of the treated animals. This delay, however, is very slight when compared with the great prolongation of the latent period previously observed,[1] after treatment with homologous leucocytes.

In the same report, it was shown that foreign leucocytes are much less toxic for monkeys. Both rabbit leucocytes and horse leucocytes can be safely injected into the meningeal cavities of these animals.

The study of the therapeutic control of meningeal tuberculosis in monkeys is made difficult by extra-dural leakage, when the inoculations and treatments are made by the method of lumbar puncture. The inoculations and treatments, in the later experiments herein reported, were therefore made through a permanent wax-trephine[2] opening in the skull.

The injection of foreign leucocytes into the meningeal cavities of monkeys, either simultaneously with the inoculation with tubercle bacilli, or subsequent to the inoculation has thus far given no definitely positive prophylactic or curative effects. In a small group of monkeys, however, inoculated and treated by the method of lumbar puncture, the repeated injection of rabbit leucocytes was associated with a considerable prolongation of the latent period in one of the treated monkeys, and by a complete prevention of the subsequent tuberculosis in a second monkey. The work with monkeys is being continued.

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[1] *Jour. Exp. Med.*, 1912, XV, pp. 1–13.

[2] *Jour. Exp. Med.*, 1912, XV, p. 3.

3 (699)

The importance of calcium in relation to growth.

By FRANCIS H. MCCRUDDEN.

[*Rockefeller Institute Hospital, New York.*]

In certain cases of retarded development there is faulty skeletal development and disturbed calcium metabolism. The bones are frail and easily fractured; large quantities of calcium are lost through the feces, and the urine is almost free from calcium. It seems probable that the retarded skeletal development is due to the lack of calcium salts available for bone growth. Other cases of retarded development show no such disturbances of calcium metabolism and the bones are of normal solidity. In these cases there is a more fundamental absence of the "tendency to grow" rather than any lack of material for growth.

4 (700)

The pyramid tract in the Canadian porcupine (Erethizon dorsatus Linn.).

By SUTHERLAND SIMPSON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

The nerve fibers of the pyramid tract pass caudalwards from their cells of origin in the motor area of the cerebral cortex and are homo-lateral until the lower part of the medulla oblongata is reached. At this level, in the majority of mammals that have been examined, most of the fibers decussate and take up a position in the lateral column of the spinal cord on the opposite side, just ventral to the posterior horn, forming the crossed lateral pyramid tract. A few fibers remain uncrossed and are found in the lateral column on the same side, constituting the direct lateral pyramid tract.

In man and the anthropoid apes a second uncrossed tract is found in the ventral column—the direct ventral pyramid tract. In mammals lower in the scale than the anthropoids, it is generally held that this direct ventral tract is not represented at all.

This disposition of the pyramid tract fibers is not found in all mammals however. In the guinea-pig (v. Bechterew, Reveley), mouse (v. Lenhossek), rat (Flechsig, King) and squirrel (Weigner), and in the monotremes and marsupials (Kölliker and Ziehen), the crossed fibers run in the dorsal and not in the lateral column of the cord.

In the spring of the present year, I obtained some full-grown porcupines with the object of investigating the course of the pyramid tract fibers in this animal. The left motor cortex was located by electrical stimulation and then extirpated in the usual way. At the end of about a fortnight after the operation in each case the animal was killed, the brain and cord removed, stained by the Marchi method and sectioned at all levels. A full description of the resulting degenerations will be published later; in this preliminary communication only the most important points will be mentioned.

In the internal capsule, pes pedunculi, pontine bundles and upper levels of the medulla oblongata, the fibers occupy the usual positions and no special comment is called for, but in the lower part of the medulla oblongata the arrangement is peculiar, and nothing similar, so far as I know, has been hitherto described in any other animal.

At the decussation of the pyramids most of the degenerated fibers cross the median raphe, pass backwards through the gray matter, and take up a position in the dorsal column of the opposite side occupying the ventral portions of the fasciculi of Goll and Burdach. A few of the crossed fibers curve outwards and enter the opposite lateral column of the cord. A very considerable number of fibers, however, remain uncrossed and are continued into the cord, forming a comparatively large and compact bundle in the ventral column extending along the margin of the anterior median fissure. Some uncrossed fibers are also found in the dorsal column of the same side but these are very scanty.

In this animal, then, the fibers of the anterior pyramid on entering the spinal cord are divided into four fasciculi, two crossed and two direct, viz: the crossed dorsal pyramid tract, the crossed lateral pyramid tract, the direct ventral pyramid tract, and the direct dorsal pyramid tract. Of these the crossed dorsal and

direct ventral tracts are much larger than the other two and can be traced as far as the lower sacral segments. The crossed lateral and direct dorsal seem to disappear in the upper thoracic region. Arranged according to the number of fibers which they contain, the order is crossed dorsal, direct ventral, crossed lateral, direct dorsal.

5 (701)

Interpolated extra-systoles, of frequent occurrence, in an otherwise normal human heart.

By M. DRESBACH and S. A. MUNFORD.

[*From the Physiological Laboratory, Cornell University, Ithaca, N. Y.*]

In the extensive literature upon extra-systoles about twenty writers have described the type known as the interpolated beat of the ventricle. Although such beats have frequently been produced experimentally, their occurrence clinically is comparatively rare.[1] Almost without exception they have been observed in cases showing gross lesions of the heart. The present case is of interest because the interpolated contractions occur persistently and frequently in a heart which, aside from a slow sinus rhythm, is otherwise normal. It furnishes a striking example of an organ on the borderland between a physiological and a strictly pathological condition.

T., a Chinese student at Cornell University, is in good health. He is able to take vigorous exercise, such as running, tennis, etc. About two years ago a cardiac irregularity was discovered in his routine physical examination. Prior to that time he was wholly ignorant of any disturbance in his heart, and is at no time conscious of the heart's contractions. Polygraphic records have frequently been made during the past two years and the organ has been found beating normally on two occasions only, and but for a short time.

The tracings show extra-systoles of the ventricle which occur with considerable regularity on some days, but usually they appear at varying intervals. The normal rate of the dominant rhythm

[1] Mackenzie, Lewis, Wenckebach, and others.

averages about 60. It may go as low as 50 during rest. The extra-systoles average about 35 per minute during rest and 45 after vigorous exercise. The ectopic beat follows the normal contraction by a period averaging ½ second. The pause following the extra-systole is variable; occasionally it is fully compensatory. The dominant rhythm is but slightly disturbed, as a rule. Often it is strikingly constant, and it is seldom more variable than the rhythm of many normal hearts. This is remarkable in view of the fact that in the tracings there is good evidence of retrograde contractions of the auricle which beats prematurely in response to the heterogenetic stimulus. Evidence for the retrogression is found in the absence of the "a" wave at certain cycles. In place of the "a" wave there is often seen a wave which precedes the normal auricular systole by 3/30 to 5/30 of a second. This premature wave is also preceded by a wave practically synchronous with the extra-systole of the ventricle.

The interpretation of the curves has been uncertain because of the unusual character of the phlebograms and cardiograms and because of the difficulty in establishing proper time relations. While several explanations may be offered for the type of irregularity exhibited, the evidence points to the one given as plausible, but not altogether satisfactory.

Physiologically this heart is interesting because of the following characters:

1. It very rarely has periods in which its action is absolutely normal. These periods are of short duration.

2. The normal sinus rate is slow.

3. Functionally the heart meets all ordinary demands made upon it.

4. There is a bathmotropic disturbance which causes persistent interpolated extra-systoles.

5. The number of extra-systoles is not decreased, except slightly in a few tracings, by an increase in the heart rate due to exercise. The tendency is for the number to rise with acceleration of the normal beat.

6. The pause following the extra-systole is variable. Occasionally it is compensatory.

7. There is strong evidence that the extra-systole of the

ventricle is often followed by a premature beat of the auricle. When the evidence of such retrograde contractions is best, the indicated time of backward conduction is 7/30 to 8/30 of a second.

8. There is no evidence of lengthened *As–Vs* intervals except at the extra-systoles.

9. There is a fairly fixed time relation between the normal and abnormal ventricular systoles.

10. There is often a considerable delay in the appearance of the carotid pulse resulting from the extra-systole.

11. There is a marked lack of synchronism between the carotid pulse and the "c" wave. The latter may precede the former by 1/20 of a second.

12. The site of the abnormal stimulus is either in the ventricle or some part of the conduction system, *e. g.*, the node, or some lower point.

6 (702)

On the differences in the effects of stimulation of the two vagus nerves on rate and conduction of the dog's heart.

By ALFRED E. COHN.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

The effects obtained in the action of dogs' hearts on stimulating the peripheral stumps of both vagus nerves were studied in fifty-four experiments. The dogs were anesthetized with ether without adjuvant, artificial respiration was maintained by the Meltzer-Auer method, and registration was accomplished by the galvanometric method. When the chest was opened, curves of auricular and ventricular contractions were also inscribed. Faradic stimulation was employed. The secondary coil of an inductorium, fed by a 2-volt dry cell, placed arbitrarily at 50 mm., was the source of the current.

On stimulating the right vagus nerve, the usual effect was obtained; both auricles and ventricles ceased to beat, generally throughout the period of stimulation. Occasionally the ventricles escaped from inhibition, but then the impulse to contraction arose

and spread in an abnormal manner; the structures normally concerned with these functions remained inhibited.

When the left vagus nerve was stimulated in the same dog, with a current of the same strength, a difference from the effect of stimulating the right nerve was observed in 88 per cent. of the fifty-four experiments. The auricles did not cease to beat, they were merely slowed,—sometimes 100 or more beats. In one group, normal ventricular contractions ceased entirely in twenty-four cases. In a second of twenty-four other cases, a ventricular contraction followed every second, occasionally every third, fourth or more auricular beats, the mechanism being one of incomplete dissociation. In a third group, the only effect of stimulation was an increase in the time occupied in conduction from auricles to ventricles.

In the first group when the left vagus was stimulated, as has been stated, normal ventricular activity ceased, but abnormal activity occasionally continued. The rate of the abnormal ventricular contractions differed from that of the slowed auricles and complete *As–Vs* dissociation resulted. Similar ventricular activity occurred also when the right vagus was stimulated, but the auricles, as is usual, ceased to contract.

The explanation offered for the phenomena occurring on left vagus stimulation is that the main effect consists in depressing the conduction system. In the first group, the ventricles cease to beat, because, on account of the great depression of conduction, they receive no impulses from the auricles. If the ventricular muscle is irritable, abnormal stimuli may be formed and an idioventricular rhythm result. When a slighter degree of depression of conduction has taken place, every second or third beat passes along the *A–V* bundle and initiates a ventricular contraction. A still slighter degree of depression results in a mere lengthening of the time of conduction.

The mechanism which results from stimulating the right vagus nerve and the negative chronotropic effect of the left nerve are not discussed at present.

7 (703)

The creatine content of muscle under normal conditions. Its relation to the urinary creatinine.

By VICTOR C. MYERS and MORRIS S. FINE.

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

Though an unusual amount of attention has been devoted to urinary creatinine since the introduction of Folin's simple colorimetric method for its estimation in 1904, relatively little consideration has been given to the supposedly related muscle creatine. The few figures which have been published giving the content of muscle creatine have simply served to indicate that the creatine content of vertebrate muscle is in round numbers .4 per cent., though figures for different animals have shown individual results varying from .3–.5 per cent. We have estimated the creatine concentration of muscle in a number of the common laboratory animals, rabbit, dog, cat, guinea pig, monkey, and likewise in man, in a few instances, where we have been able to obtain good samples at autopsy. Our muscle analyses on 20 normal rabbits and 5 dogs have yielded very uniform figures and indicate that the creatine concentration of rabbit muscle is .52 per cent. and of the dog muscle, .37 per cent. Our data on the muscle of the other species are as yet insufficient to warrant special comment. This uniformity recalls the constancy of the daily creatinine elimination first noted by Folin.

It is a curious fact that the creatinine coefficient of the rabbit is fully a third higher than that generally found in man and various experimental animals, dog, pig, and guinea pig. In forty rabbits, the average coefficient has been found to be 14.3 while for a healthy man, the normal coefficient is about 9, and for the dog, the average is probably a little lower (for 3 animals it was found to average 8.4). Comparing these values with those for the creatine concentration of the muscle (rabbit .52, man .39 and dog .37 per cent.), an interesting, and, as we believe, more than an accidental relationship is revealed.

In view of the lack of proof of the connection of creatine and creatinine in metabolism, our original experiments were planned in an endeavor to ascertain whether in a given animal, in this case the rabbit, there was a constant relationship between the total creatine of the body and the daily creatinine. This ratio was ascertained in a series of one growing and ten adult rabbits. It was not absolutely constant, and appeared to vary with the weight of the animal, being 53.3 : 1, in an animal of 1.39 kilos and 44.7 : 1 where the weight was 2.13 kilos. For five animals of nearly the same weight, 1.9–2.1 kilos, comparatively constant ratios, 44.7, 45.0, 44.4, 44.4, and 44.9 : 1 were obtained.

In a further attempt to ascertain any relationship between body creatine and urinary creatinine, the figures for the creatine concentration of the body were arranged in order of the creatinine coefficients. The first five adult animals with an average coefficient of 13.6 had an average body creatine concentration of .170 per cent., while the second five animals with an average coefficient of 15.0 had a creatine concentration of .193 per cent.

Our fasting experiments below indicate that the amount of creatine remaining in the body after starvation bears a relationship, not only to the amount of creatine lost in the urine during this period, but also to the amount of the creatinine elimination.

An interesting observation with regard to the creatinine, in the series of eleven normal animals, is that the amount of its average daily elimination followed that of the body weight in every case. This is in accord with Folin's original statement that body weight appears to be the most important factor in determining the amount of its elimination.

It is not supposed that these experiments offer any definite proof of the origin of the urinary creatinine from muscle creatine or some common precursor substance, but in view of the fact that no data have been adduced to show such a relationship, these experiments may be of value as advancing this hypothesis.

8 (704)

The creatine content of muscle during starvation and its relation to urinary creatine.

By VICTOR C. MYERS and MORRIS S. FINE.

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

There has recently arisen some little discussion with regard to the creatine concentration of muscle during starvation. Data have been submitted to show that inanition in the rabbit results in an increase in the content of muscle creatine, and it has further been argued that there is an actual increase in creatine formation. In other quarters, it has been claimed that starvation (experiments with the dog) produces a marked decrease in the percentage content of muscle creatine.

From nine experiments which we have already completed on starving rabbits, we can agree with both of these observations as regards an increase or a decrease in the content of the muscle creatine. In three experiments, a decided increase in the content of creatine in muscle has been found; in one the per cent. of creatine was normal, while in five it was even more strikingly below normal. In addition to the increased or normal creatine content of muscle, the first four animals mentioned showed an increased percentage content of creatine in the body. This we are not inclined to ascribe to an increased creatine formation.

The reason for this increased or decreased content of body and muscle creatine appears to be satisfactorily explained by our results. The animals having an increase in the content of muscle creatine eliminate a comparatively small amount of creatine in the urine, while those in which the muscle and total body creatine has been considerably depleted, eliminate an equally large amount of creatine in the urine. In other words, the content of muscle creatine during starvation is dependent upon the amount of and the rate of creatine excretion in the urine. Just why some animals eliminate creatine more rapidly than others, we are as yet unable to explain, although we assume that this is connected with the length of the fast and the state of nutrition of the animal. The

rate of the loss of creatine appears to closely parallel that of the total nitrogen.

Still an added factor in this loss of creatine from the body is the excretion of creatinine. In experiments where the creatine of the urine plus that of the tissue does not entirely account for the creatine which should normally be present in the body, it is found that a considerable amount of creatinine has been eliminated in the urine, *e. g.*, in the case of a comparatively long fast. When this creatinine in terms of creatine is added to the creatine of the tissue and urine, this total exceeds the total normal body creatine by about 10 per cent., this excess probably representing the amount of creatine and creatinine formation. It would seem probable from these data that during starvation, the creatine storehouse was depleted not only by a loss of creatine in the urine, but also by the loss of creatinine.

It has been assumed for some time that the creatine appearing in the urine during starvation and in various pathological conditions was derived from the creatine of the muscle, and measured the amount of muscle disintegration, though so far as the authors are aware this point has never been conclusively demonstrated. In our experiments, we have found that when the weight of the creatine excreted in the urine was added to the weight of creatine still remaining in the body after the period of starvation, the amount of creatine was only slightly below that which would have been found in the body had the animal been killed prior to starvation. This would seem to demonstrate that the creatine appearing in the urine in starvation was derived from the creatine of the muscle.

9 (705)

Reversal of the cardiac mechanism.

By HORATIO B. WILLIAMS and HENRY JAMES.

[*From the Physiological Laboratory of Columbia University, New York.*]

The subject of investigation, H. M., came under observation at the Vanderbilt Clinic July 15, 1912. He has had a persistent

diarrhea for a year with several watery stools a day. Otherwise he has been well until three months before admission to the clinic.

During these three months and subsequently, he has suffered from attacks of dizziness of increasing severity. In one of these attacks he fainted and fell in the street. Muscular effort increases the giddiness. He has had a good deal of headache. He is very drowsy and is annoyed by numbness and tingling of the hands and feet.

Until the onset of symptoms he had been a heavy drinker. He denies lues and gonnorrhea. The Wassermann test was negative. The blood and urine are normal. The heart is not enlarged. The sounds are faint but clear, and there are no murmurs. The third sound has been distinctly audible at most examinations and has been recorded graphically. The pulse is regular, 40 per minute. Systolic bloodpressure varies between 95 mm. and 110 mm. and diastolic pressure is usually about 65 mm. The electrocardiograms show that the heart beat is initiated by the ventricles, the auricular beat succeeding the ventricular after the usual conduction interval. This is evidenced by the presence of an inverted *P* between *R* and *T* in leads II and III. In lead I, *P* is of very small amplitude, but can be clearly distinguished on close inspection and stands in the same relation to *R* and *T* as in the other leads.

This interpretation of the electrocardiograms has been confirmed beyond reasonable doubt by examination with Roentgen rays and fluoroscope. The contractions of the auricle can be distinctly seen.

We are not yet ready to offer an explanation for the peculiarly slow intrinsic rhythm of the auricles which permits the ventricle to initiate the beat. Neither atropin nor digitalis has any measurable effect on the condition. The subject is still under observation.

10 (706)

The rôle of lipoids and particularly lecithin in narcosis.

By B. KRAMER.

[*From the Physiological Laboratory of Cornell University Medical College, New York City.*]

Following the discovery of anesthetic properties of ether by Jackson and Morton in 1846, numerous theories appeared in the literature which aimed to explain this important phenomenon. Of these the well-known Meyer-Overton theory is the only one that has survived the test of time. It reads as follows: "The narcotizing substance enters into a loose physio-chemical combination with the lipoids of the cells, perhaps with the lecithin, and in doing so changes their normal relationship to other cell constituents through which an inhibition of the entire cell chemism results." Evidently this theory casts no light upon the nature of the alterations in cell chemism that follow. It remained for Verworn, Mansfeld, Bücher and Heaton not only to demonstrate the nature of these changes in cell chemism but also to show that the anesthetic state itself is in all probability dependent upon these alterations.

Reicher, who demonstrated the constant presence of lipoidemia after narcosis, explained this as being a protective mechanism; the lipoid molecules acting as amboceptors uniting with anesthetic and thus protecting the more vitally important brain lipoids. Nerking attempted to prove this experimentally by injecting various quantities of a 1 to 20 per cent. lecithin emulsion in normal salt solution intravenously, intraperitoneally, intraspinally or subcutaneously in animals which had been narcotized or were about to be anesthetized and claimed to have shown that "The injection of lecithin has an undoubted influence upon the duration and after effects of anesthesia in that it shortens its duration, brings about a more rapid return to consciousness and eliminates unpleasant after effects."

A careful analysis of Nerking's work shows it to be scarcely worthy of serious consideration, owing to its lack of exactness in the dosage of the narcotic and the indefiniteness of the criteria used.

Nevertheless, the importance of his conclusions, if confirmed, is quite evident if this could be done with the above mentioned experimental defects eliminated.

In order to accomplish this all anesthetics were given intravenously, a 5 or 10 per cent. ether solution in normal salt solution being used, and this was injected at definite rates; the lecithin emulsion was also injected directly into the vein. The criteria used were the appearance and disappearance of the corneal reflex, or, when this did not occur, the loss of cutaneous pain sensation was used. Other criteria were the time when the animal first raised its head, when it first assumed the erect posture, and when all ataxia disappeared. The results obtained seem to point overwhelmingly to the following conclusions:

1. That the intravenous injection of 5 to 30 c.c. of a 5 or 10 per cent. emulsion of lecithin, depending upon the size of the animal used, does not interfere with the induction of anesthesia and that this can be accomplished as readily in animals thus injected as in controls.

2. That in the majority of experiments, lecithin has no effect upon the rapidity with which the various phenomena which indicate the animal's recovery from the effects of anesthetic appear, in fact, in most cases, it retards their appearance.

The above experiments do not seem to bear out Reicher's assumption as to the cause of the lipoidemia and the explanation of this phenomenon still remains an open question.[1]

11 (707)

Habit and its relations to the nervous system in the earthworm.

By ROBERT M. YERKES.

[*From the Psychological Laboratories, Harvard University.*]

This is a preliminary report of an investigation now in progress, the purpose of which is (*a*) to demonstrate whatever ability the earthworm may have to acquire habits of a certain order; (*b*) to discover the characteristics of any habits which appear; (*c*) to

[1] The complete report of this work will appear in the *Journ. of Exp. Med.* for February, 1913.

enumerate and evaluate the various external and internal influences on habit-formation; (*d*) to ascertain the degree of permanency of the habits and (*e*) to discover their relations to the anterior ganglia (brain).

By means of a T-shaped maze constructed from plate glass, specimens of the manure worm, *Allolobophora fetida*, were tested. The maze was placed with the stem directed toward the light. Across one of the arms a piece of sandpaper was placed and, just beyond it, a pair of electrodes. The other arm was left open so that the worm might escape to an artificial burrow. The worms were driven into the T by light and the chief motive for escape therefrom was the tendency to avoid light. It was the purpose of the test to demonstrate (*a*) any ability which the manure worm may possess to acquire a direction habit and (*b*) to associate the tactual experience of contact with sandpaper with the electrical shock which regularly followed the tactual stimulus in case the worm continued to move forward after reaching the sandpaper.

Trials were made in daily series varying in number from five to twenty. The five-trial series were found, on the whole, most satisfactory.

Referring now exclusively to the results obtained for a single worm which has been under observation since October, 1911, the following data may be presented. (1) *Allolobophora* is capable of acquiring certain definite modes of reaction. (2) Modifications appear as the result of from twenty to one hundred experiences. (3) The behavior is extremely variable because of variations in external conditions and in the condition of the worm itself. (4) There is a tendency to follow the mucous path through the apparatus but this is not sufficiently strong or constant to yield perfect results. (5) The following are the chief modifications which have been noted: (*a*) Increased readiness to enter the apparatus and to desert it for the artificial burrow; (*b*) apparent "recognition" of the artificial burrow which is used as "exit tube"; (*c*) a gradual increase in the number of avoidances of the sandpaper and of contact with the electrodes as a result of the "warning" influence of the sandpaper; (*d*) the disappearance of the early tendency to retrace the path through the stem of the T; (*e*) the similar disappearance of the tendency to turn back after progressing well

toward the exit tube. (6) The correct performance of a thoroughly ingrained habitual act, of the kind studied in this investigation is not dependent upon the "brain" (portions of the nervous system carried by the five anterior segments), since the worm reacts appropriately within a few hours after its removal. (7) As the "brain" regenerates, the worm exhibits increased initiative, its behavior becomes less automatic, more variable. (8) Within four weeks after the operation the regenerated segments appear superficially complete and the worm naturally burrows in a mixture of earth and manure. (9) Two months after the removal of the "brain," during the last four weeks of which period no training was given, the habit had completely disappeared from worm No. 2, the subject to whose responses this paper is devoted, and in its place there appeared a tendency to turn in the opposite direction to that demanded in the training. (10) Systematic training for two weeks resulted in the partial re-acquisition of the original direction-habit.

The general results which have just been stated are subject to modification in the light of additional data. To the experimenter, it seems that the particular individual which has been longest under observation is in many respects exceptional. It is perfectly clear, however, from results obtained with six individuals that important modifications in behavior appear as the result of training. It is equally certain that direction-habits are not readily acquired.

It is the purpose of the experimenter in the continuation of the investigation to pay especial attention to the relation of the nervous system to modifications of behavior.

12 (708)

Preliminary note on the action of glucose upon the amount of epinephrin in the blood.

By ISAAC OTT and JOHN C. SCOTT.

[*From the Laboratory of Physiology, Medico Chirurgical College, Philadelphia.*]

The modern theory of experimental diabetes is that a glycosecretory center is located in the medulla, from which impulses

pass down the cord, emerge in the splanchnics, and go to the liver to increase the transformation of glycogen into glucose. It is held by some that as the splanchnics contain the secretory fibers of the adrenals, that these impulses from the glyco-secretory center increase the amount of epinephrin, which mobilizes the glycogen of the liver and thus produces a diabetes. It is well known that epinephrin is a stimulant of the sympathetic nerves, hence it is a stimulant of the splanchnics. We have found the injection of glucose per jugular in the cat increases the amount of epinephrin in the blood, as shown by the intestinal strip of the rabbit. Hence we have here a circle: epinephrin stimulates the secretory nerves of the adrenals to produce epinephrin, which via the glycogen of the liver produces more glucose, which in its turn generates more epinephrin. We have also found the other sugars to increase the amount of epinephrin in the blood. Pilocarpin, skatol and indol also augment the amount of epinephrin in the blood.

13 (709)

Preliminary note on the inhibitory and synergistic hormones of the secretion of milk.

By ISAAC OTT and JOHN C. SCOTT.

[*From the Laboratory of Physiology, Medico Chirurgical College, Philadelphia.*]

We have studied on the goat the hormones which inhibit the milk secretion. We have found that the ovary inhibits the action of infundibulin, pineal gland, corpus luteum and thymus upon the secretion of the mammary gland. Pancreas, spleen, iodothyrin-parathyroid and adrenalin also inhibit the action of indundibulin. Orchitic extract increases the activity of infundibulin, thus being a synergistic agent. Egg albumen does not inhibit the action of infundibulin.

14 (710)

Heterochromosomes in mammals.

By H. E. JORDAN.

[*From the Department of Anatomy, University of Virginia.*]

Heterochromosomes have now been reported for the male germ cells of the following mammals: man and rat (Guyer, '10); armadillo (Newman and Patterson, '10); opossum (Jordan, '11); guinea-pig (Stevens, '11); and bat (Jordan, '12). Winiwarter and Sainmont, '09, report a longitudinally split "monosome" in the oöcyte of the cat.

A comparative study of mammalian spermatogenesis reveals the absence of typical heterochromosomes in mongoose,* cat, squirrel, rabbit and pig. Heterochromosomes are clearly present at synapsis and prophase in the primary spermatocytes of the following forms: white mouse, sheep, horse, mule, dog and bull. Regarding dog, rabbit, and the monkey, the evidence is not yet decisive.

At certain stages the heterochromosomes (chromosome-nucleoli) appear single (accessory; monosome), at others double or bipartite. The latter appearance suggests a pair of idiochromosomes; but the body is more probably a split accessory.

The absence of discernible heterochromosomes in the male, and their conspicuous presence in the female, of the cat indicates their presence in one or the opposite sex in all forms. If this hypothesis can be supported by evidence from the oöcytes of mongoose, squirrel, pig, rabbit and similar forms, cogent additional confirmation is given to the idea of a special significance of heterochromosomes, probably in connection with the determination of sex.

A simple explanation of sex-determination suggested by these and other facts — and one in apparent accord with a large body of experimental and cytological data — would seem to be to regard

* This material was collected at the temporary Marine Biological Station of the Carnegie Institution of Washington at Montego Bay on the expedition to Jamaica, B. W. I., in February and March, 1912, under the directorship of Dr. Alfred G. Mayer.

the heterochromosome-complex or "X-element" (Wilson), contributed by the spermatozoön, as an inhibitor to male sex. Regarded in terms of Mendelian concepts, however, an apparent contradiction results in that the *presence* of a determiner (inhibitor to maleness) would here have to be recessive to its *absence*. But in terms of a quantitative interpretation two X-elements in the zygote would prevent, one X-element permit, the development of male sex. Similarly with respect to the phenomenon of sex-limited heredity: the X-element may act as the inhibitor in the female to the male-limited character.

15 (711)

A comparison of chemical with microchemical methods for the determination of varying amounts of glycogen in the liver.

By G. Y. RUSK.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

In collaboration with Dr. F. P. Gay, a study has been made of the glycogen in the livers of 22 rabbits, comparing the chemically determined amount with the histological appearance with a view to finding the value of the latter for comparing small differences in glycogen content. Pflüger's method was employed as far as the conversion of glycogen to glucose and for the quantitative estimation of the latter Bertrand's method was used. For the histological picture, Best's carmine method and Langerhans' modification of Ehrlich's iodine method were employed.

The following table gives the comparative results. The chemical factors are reduced to a common denominator, viz., the amount of copper which is reduced by 100 gm. of liver, and arranged from the highest to lowest amounts. The histological results are placed in a parallel column. While in the main there is faily close correlation, yet there are two striking discrepancies (No. 23 and No. 24).

This study presents, so far as we can determine, the first attempt to correlate chemical and microchemical findings with a view to utilizing the latter for comparing slight differences of glycogen in the liver. The results do not warrant the assumption

of parallel results between the chemical and microchemical results; hence the histological method is not available for accurate comparative work.

TABLE.

Liver of rabbit number	Chemical determination of glycogen (copper gms. per 100 g. liver).	Liver of rabbit number	Histological amount and distribution of glycogen.
23	13.940	20	Throughout lobule; most marked about central vein and in single layer of cells at periphery.
20	11.072	21	Very marked in inner two-thirds of lobule; trace in outer one-third.
22	5.688	22	Inner half of lobule shows well-marked glycogen; also single layer about periphery.
18	5.014	12	Much the same distribution as No. 21 but less in quantity.
12	4.172	15	Same general distribution but slightly less.
19	3.612	18	Slightly more diffuse in distribution but less intense.
21	3.609	13	Narrow border about central vein shows glycogen well marked.
26	3.098	19	Somewhat less.
47	2.604	26	Rather diffuse and pale in inner third.
15	2.380	48	Somewhat less.
28	2.371	44	In thin strip of 1 to 2 cells about central and sublobular veins is well-marked glycogen.
42	2.317	39	Trace.
43	2.161	25	Trace.
48	2.152	42	Trace.
39	1.895	28	Trace.
38	.978	47	Trace.
41	.942	43	None.
27	.836	41	None.
44	.720	27	None.
24	.560	24	None.
25	.484	23	None.
13	.366	38	None.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-first meeting.

The Rockefeller Institute for Medical Research, December 18, 1912.
President Ewing in the chair.

16 (712)

The effect of strychnin in cardiectomized frogs with destroyed lymph hearts; a demonstration.

By S. J. Meltzer.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

In several communications we have reported that the injection of solutions of strychnin, morphin or acid fuchsin in cardiectomized frogs is liable to bring on convulsions of these animals. The lymph hearts continue to beat for a while after the cardiectomy. But since the lymph hearts assist the circulation only by emptying their contents into veins, it seemed to be evident that the removal of the blood heart eliminates also the circulatory function of the lymph hearts. I have therefore assumed that the above mentioned alkaloids reach the central nervous system by way of the lymph spaces, which are connected throughout the body, and which are capable of serving as a path for distribution by means of a peripheral mechanism. In a recent paper by Abel (*Jour. of Pharmacology*, III, 581, 1912) in which our facts were confirmed and in which it was admitted that the activity of the posterior lymph hearts can not come into consideration, the statement was made that "the appearance of convulsions in the experiments of Meltzer and his pupils with acid fuchsin, morphin and strychnin depends entirely on the integrity of the anterior lymph hearts." This statement is supported by a report of experiments in which, after destruction of

the anterior lymph hearts, in addition to cardiectomy the alkaloids under discussion did not bring on any convulsions. I shall not enter here into a discussion of the entire subject. I merely wish to let you witness some indisputable facts. You see here a series of frogs from whom the thoraco-abdominal viscera have been removed, and in addition, the lymph hearts were destroyed by cauterization. All these animals were injected about an hour ago with strychnin; the injections were made in some animals into the dorsal lymph sac and in others into the femoral sacs of both thighs. You see that they respond to a tap with a tetanus. This shows definitely that the injected strychnin reached the central nervous system of these completely eviscerated frogs without the help of the anterior lymph hearts. But you see also that the trays holding the frogs are kept over ice. This is done because at the room temperature, I could not be sure of the success of my demonstration. It is possible that Abel's observations were made in May, when such experiments are apt to fail.

17 (713)

Pulmonary lesions by intra-bronchial insufflation of cultures of B. megatherium. With a demonstration.

By MARTHA WOLLSTEIN and S. J. MELTZER.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

The production of experimental pneumonia in dogs by means of intra-bronchial insufflation of bacterial cultures has now been carried out in several series of investigations. In the first series Lamar and Meltzer produced lobar pneumonia by insufflation of cultures of a virulent pneumococcus. In a second series we produced lobular pneumonia by insufflation of a virulent streptococcus and of the influenza bacillus. Besides the differences in the gross appearances of the lesions both pneumonias offered the following distinguishing points: In the lobar pneumonia of the virulent pneumococcus there was a mortality of about 16 per cent.; even in the non-fatal cases there was bacteremia present in the first twenty-four hours; the exudate was rich in fibrin and the framework of the lungs was invariably free from leucocytic

infiltration. In the cases of broncho-pneumonia produced by the streptococcus or by the influenza bacillus there was no mortality; at no time was there a bacteremia; the exudate contained very little fibrin, but the framework of the lungs was invariably and often very intensely infiltrated with leucocytes.

In a third series of experiments, which has not yet been published, we studied the effects of insufflation of a non-virulent pneumococcus. This organism also caused the development of a lesion which macroscopically resembled the lesion of lobar pneumonia produced by the virulent pneumococcus. However, there was no mortality, no bacteremia, and an exudate comparatively poor in fibrin; the framework of the lung was here again practically free from leucocytic invasion.

The results obtained with the non-virulent pneumococcus led us to a study of the intra-bronchial insufflation of such a non-pathogenic organism as the *B. megatherium*. Ten experiments were made. The insufflation brought on a definite pneumonic lesion in every case. From the gross appearance the lesion has to be considered as that of lobar pneumonia of a milder type. The entire process is essentially similar to the one caused by the non-virulent pneumococcus, but its course is even milder. The development is slower, the reaction is less intense and the resolution occurs sooner; there is practically no fibrin at all in the exudate and bacteria can only occasionally be cultivated from the exudate of 24 hours' duration. There is little involvement of the framework.

We show you here several lungs in the various stages of the inflammatory process; the lesions were produced by the intra-bronchial insufflation of twenty cubic centimeters of an eighteen hour old bouillon culture of the *B. megatherium*.

18 (714)

The development of experimental pneumonia under direct observation of the lungs in the living animal.

By MARTHA WOLLSTEIN and S. J. MELTZER.

[*From the Laboratories of the Rockefeller Institute.*]

Experimental pneumonia produced by intra-bronchial insufflation of virulent pneumococci may develop quite rapidly. Thus in the experiments of Lamar and Meltzer it was observed that in one instance seven hours after the injection, nearly complete consolidation affecting the greater part of one lobe was already present. We therefore now made several experiments in which the lungs were under direct observation continuously for several hours after the insufflation of the culture. The procedure has been as follows. The dog was anesthetized by the cone method, and a tube introduced into a bronchus as deep as it could be pushed; the pneumococcus culture was then injected. Immediately after, the tube was withdrawn, so that its lower end was just above the bifurcation, and the arrangement made for continuous intratracheal insufflation. The dog received about "half ether" and was continually under complete anesthesia. The thorax was now widely opened transversely and about two thirds of three or four of the lower ribs on the right side removed; the right lower lobe, which is usually the seat of the inflammation, was now exposed to full view. We shall not enter here upon details. It may suffice to state that *we were able to watch the successive stages from the earliest signs of engorgement to complete hepatization. We were also able to establish changes in the auscultation phenomena in the consolidated parts, in some instances even clearly suggesting tubular breathing.* We intended to demonstrate such experiments, and we prepared several animals for this purpose. The experiments were successful indeed, but we did not take the lateness of the hour of the meeting sufficiently into account. We prepared the experiments too early and all the animals died before the meeting began. The acute infection, the great loss of heat, the severe operation, and the long-lasting anesthesia are severe factors to contend with. So far five hours has been the longest

time we could keep them alive. We show here the lungs of one of these dogs. Death occurred in this instance about four hours after the injection. The quantity of the injected pneumococcus culture was quite large—about twenty cubic centimeters. About two thirds of the right lower lobe is consolidated; as you see, it is very dark and firm, and the surface shows numerous small patches of fibrin.

19 (715)

Differences in the toxic effects of ether and chloroform, as observed under intratracheal insufflation.

By T. S. GITHENS and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

In studying the toxic effects of chloroform and ether, when administered by the method of intratracheal insufflation, we observed various differences in the course of the intoxication brought on by excessive doses of these anesthetics. We shall discuss here, however, only the differences in the toxic action of these drugs upon the functions of respiration and blood pressure. At the outset we have to point out, that when administering the anesthetics by the insufflation method one of their dangerous effects is here eliminated; it is the danger which is bound to result from a partial or complete paralysis of the respiratory function. Under the method of insufflation, life remains safe even when the animal is completely curarized. Observations may be carried on, therefore, even after spontaneous respiration is completely abolished. On the other hand, intratracheal insufflation carried on with ordinary, permissible air pressure, does not cause apnea, that is, the individual continues to carry on its own spontaneous respirations, which on tracings are easily distinguishable from the infrequent partial interruptions of the continuous insufflation of air.

The observations which we wish to report here briefly are as follows. When using ether, a certain dose may be administered which is amply sufficient to keep the animal completely anesthetized, while respiration and blood pressure may remain practically

unimpaired for several hours. When this anesthetic dose is exceeded, the first striking effect is upon the spontaneous respiration which may be rapidly abolished. For instance, if complete anesthesia has been accomplished by a dose which we term "½ ether" or "¾ ether" and now "full ether" is turned on, the respiration may stop completely within one to five minutes. At this stage blood pressure is not impaired. When, however, "full ether" is continued the blood pressure begins to come down. The descent is very gradual and slow. It is rarely less than one hour, in some cases it may be even several hours, before the pressure reaches the dangerous stage. At that stage the blood pressure may not be above 20 or 25 millimeters of mercury, and the pulse pressure also considerably reduced. However, even at this stage when the ether is turned off, the pressure may begin to rise at once; although some time has to pass before the respiration returns, and there is still another interval before reflexes and consciousness return. The duration of the returning ascent is shorter than the duration of the descent. It is, however, not absolutely necessary to shut off the ether completely; a reduction to "half ether" is, as a rule, soon followed by a return to a degree of blood pressure which is sufficient to obviate danger.

The ready abolition of the respiration by ether is a practically important phenomenon; it may serve as an indication that the etherization has entered the toxic zone. It is a danger signal, and since hours may pass before the real danger will be arrived at, it is a safe and very valuable signal.

It is different with chloroform. In doses which are undoubtedly in excess of the anesthetic dose, respiration and blood pressure go down practically together, and this in a comparatively short time. When the administered dose is only slightly in excess of the reliably anesthetic dose, the impairment of respiration does not set in as early as under ether intoxication; but when this sets in, blood pressure begins to fall also, although the respiration may in some cases cease fifteen or twenty minutes before the fall of blood pressure reaches the danger point. Respiration, perhaps, resists chloroform intoxication slightly longer than that of ether, while blood pressure is affected much more rapidly and profoundly by chloroform than by ether. The zone separating the safe

anesthetic and danger lines is narrow, and there is no reliable danger sign.

20 (716)

The relation of leucocytic extract to body fluids.

By WILFRED H. MANWARING.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

In a previous communication[1] it was shown that a comparatively strong bactericidal substance can be extracted from horse leucocytes. This substance loses its bactericidal power, in whole or in part, if it is mixed with homologous or foreign sera, with pathological exudates, with cerebro-spinal fluid, with the products of aseptic tissue autolysis, or with most of the products obtained by the bacterial decomposition of tissues. It also loses its bactericidal power if it is mixed with the products obtained by the prolonged autolysis of leucocytes themselves.

With sera that are in themselves bactericidal, not only is there a loss of the bactericidal power of the leucocytic extract in such mixtures, but there is also a destruction or inhibition of the bactericidal power of the serum itself. This gives the phenomenon of two bactericidal substances, an active serum and an active leucocytic extract, added to each other, producing a non-bactericidal substance, a good culture medium for bacteria.

An analysis of the antibactericidal action of serum shows that it is due to the combined effects of three factors: (1) the antibactericidal power of the serum colloids, (2) the antibactericidal power of sodium chloride and the other neutral diffusible serum components, and (3) the antibactericidal action of the diffusible serum alkalies.

Alkalies are very strongly antagonistic to the leucocytic bacteriolysin. The addition of 1/200 per cent. NaOH to leucocytic extract is usually sufficient to completely inhibit its bactericidal action. Acids, on the other hand, apparently have little or no antibactericidal effect.

This antibactericidal power of serum and tissue fluids can not be overcome by increasing the amount of leucocytic extract in the

[1] These PROCEEDINGS, Vol. IX, 1912, p. 74.

mixtures, except when the serum and tissue fluids are tested in minute quantities or greatly diluted. If tested in the full strength in which they appear in the animal body, no amount of leucocytic extract added to them is able to overcome or to exhaust their antibactericidal power.

Attempts to lessen the antibactericidal power of serum and body fluids by neutralizing their alkalinity with boric acid, acetic acid and other weak acids, are occasionally partially successful. Acidulation of the non-bactericidal mixtures occasionally restores part of their bactericidal power. In no experiment thus far done however has more than a quarter of the original bactericidal power been restored by this means.

The mechanism of the antibactericidal actions of serum and tissue fluids has not been determined.

21 (717)

On the lysis of tubercle bacilli.

By WILFRED H. MANWARING and J. BRONFENBRENNER.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

It was pointed out by Koch, and has since been confirmed by others, that an animal suffering from a chronic local tuberculous lesion is more resistant than the normal to inoculation with tubercle bacilli.

This heightened resistance has been studied by numerous workers. Calmette, for example, found that tubercle bacilli, injected into the subcutaneous tissues of tuberculous cattle, soon showed marked involution and degenerating forms, but that the bacilli did not completely disappear from the site of the inoculation and the neighboring lymph glands by the end of 120 days. A rapid lysis or destruction of the bacilli was not observed by these workers.

Recently Beycke and Much[1] and others have applied the Pfeiffer

[1] Beycke and Much, *Beitrag z. Klinik f. Tuberk.*, 1910, XV, p. 277.
Much and Leschre, *ibid*, 1911, XX, p. 405.
Kraus and Hofer, *Deutsch. med. Wochenschr.*, 1912, XXXVIII, p. 1227; *Wiener klin. Wochenschr.*, 1912, XXV, p. 1112.

technique to a study of this phenomenon. They have found that tubercle bacilli, injected into the peritoneal cavities of tuberculous guinea pigs, undergo rapid degeneration and a rapid decrease in number.

They interpret these changes as a rapid lysis of bacilli. If this interpretation is correct, it will necessitate a revision of current ideas concerning the resisting power of the tubercle bacillus.

We have repeated and extended their work. We have found that tubercle bacilli, injected into the peritoneal cavities of tuberculous guinea-pigs, will occasionally give the degeneration forms and the non-staining forms they describe; and that, under certain conditions, there may be a complete disappearance of the bacilli from the peritoneal fluids within as short a period of time as three hours.

Whether this disappearance is due to an actual lysis of the tubercle bacilli, or to other causes, we have not yet determined. As evidence in favor of lysis we have the observation that all of the normal control guinea pigs injected intraperitoneally with the test suspensions of tubercle bacilli have died from a fulminating type of visceral tuberculosis, within a period of from three to four weeks, while most of the tuberculous guinea pigs, receiving the same test doses, have survived for a longer period of time. A few of these tuberculous guinea pigs however have died within twenty-four hours after the intraperitoneal tests, suggesting an anaphylactic reaction.

We have obtained a similar rapid disappearance of tubercle bacilli from the peritoneal cavities of tuberculous rabbits, from tuberculous rats, and from tuberculous dogs. The mechanism of the phenomenon is now under investigation.

22 (718)

The rôle of phagocytosis in involuting organs.

By MAX MORSE.

[*From the Boardman Laboratories, Trinity College, Hartford, Conn.*]

In order to clear the way for a study of the transfer of protein in the involuting tail of the tadpole, it was necessary to reëxamine the rôle which has been ascribed by Metschnikoff, Barfurth,

Bataillon, Mercier and many others to the leucocytes in producing the phenomenon. Looss believes on histological ground, that if leucocytes play any part in the process of tissue atrophy and absorption, they play a minor and secondary one rather than a primary rôle. The process of atrophy begins prior to the invasion of the leucocytes into the muscle of the tail and this has been described by Mercier and others, who hold that phagocytosis is the principal factor. Moreover, it has been described for other involuting organs, such as in the metamorphosis of insects, the absorption of the gills of amphibia, etc. It has been suggested for the involution of the mammalian uterus, likewise, so that it may be said that investigators are in accord in observing a dissolution of the muscles and other tissues in atrophying organs prior to the advent of leucocytes.

It is to be expected that if phagocytosis plays any important rôle in the inception of the process of absorption of tissue in the larva of the frog, the blood would show an increase in the total number of leucocytes during the stages of metamorphosis and moreover there would be an increase in polymorphonuclear leucocytes during these stages, to compensate for the drainage of these cells into the muscles. In order to examine this point, smears were made of the blood from the larvæ of the bull-frog, *Rana catesbiana* and from the western pickerel frog, *Rana areolata*. Thirty specimens were used, the blood being permitted to flow into a capillary tube from a lesion in the heart and then blown upon a slide, dried in the air and stained with Wright's stain. A large number of hematocytometer counts were made upon fresh blood, but this method of estimation was abandoned on account of the unsurmountable difficulty of recognizing the different kinds of young corpuscles. As Freidsohn has shown, the various sorts of leucocytes together with the erythrocytes take their origin from cells more or less similar in appearance in the earlier stages, this common stage resembling the large mononuclear leucocyte of the human blood.

The following table gives the summary of the differential counts made upon the smears:

Polymorphonuclear leucocytes are in slight advance in absorbing individuals over those not yet metamorphosing, but there is a

	Polymorpho-nuclear, per cent.	Basophiles, per cent.	Eosinoph., per cent.	Large M., per cent.	Small M., per cent.
Absorbing	9.8	4.2	6.5	36.1	42.4
Non-absorbing	8.6	4.7	7.0	20.6	59.0
Adults	18.3	6.2	0.4	13.2	61.2

much greater number of these cells in the adult. Of basophiles, there are more in the adult than in the larval stages and more in the non-absorbing than in the absorbing animals, although the difference is slight. Eosinophiles occur in about the same relative numbers as the basophiles, but they are rare in adults. The case is more difficult to analyze in the large and small mononuclear types, for doubtless these classes have been made to include young leucocytes of the foregoing kinds. Inasmuch, however, as histological sections of the tail of the metamorphosing larva show few cells of either of these classes, it is permissible to rule them out of the consideration as phagocytes.

From this evidence, it seems fair to conclude that phagocytosis is not the primary factor involved in the involuting tadpole's tail and, since the process of involution everywhere involves a breaking down of tissue before leucocytes have entered the tissue, it may be concluded that these blood cells nowhere are of importance in explaining the process of tissue absorption. Exception may be made in the case of certain types of carcinoma, which, however, have not been satisfactorily studied from this point of view.

Evidence will be presented later that the process of involution concerns autolysis, as has been suggested in the case of mammalian tissues. Evidence will also be submitted to show that the transfer of tissue involves a closed circuit and that there is no increase in total nitrogen in the excreta during the process of metamorphosis.

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23 (719)

Effect of phlorhizin on a dog with Eck fistula.

By **N. B. FOSTER.**

It has been stated by Rosenfeld that the administration of phlorhizin to dogs with Eck fistula does not induce glucosuria. This observation would have so much bearing upon our ideas of the mode of action of phlorhizin that the subject required confirmatory evidence.

One gram of phlorhizin in olive oil emulsion was given to a dog on which an Eck fistula had been done. Glucose was found in the urine in considerable quantity for nine days subsequent to the phlorhizin adminstration.

24 (720)

On elimination through the mucosa of the urinary bladder.

By **ISRAEL S. KLEINER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In a previous communication (*Journal of Exper. Medicine*, XIV, 274, 1911), I stated that after intravenous injections of large amounts of dextrose the intestine contained on the average 1.2 per cent. of the injected amount of destrose and in nephrectomized animals the dextrose content of the intestine reached the average of 2.2 per cent. The question arose then whether in the presence of a strong hyperglycemia, and especially after double nephrectomy, all mucous membranes are slightly permeable to this substance. The presence of measurable quantities of dextrose in the gastro-intestinal canal is no evidence for the permeability of the mucosa as such, since the mucous membrane of this organ is studded with numerous glandular structures which may readily

be instrumental in the removal from the blood of substances present there in excess. In the experiments I wish to communicate here, I selected, therefore, the bladder for the study of this problem since the mucous lining of this organ is practically the only membrane which possesses no glands. The rabbit was the experimental animal used.

After performing a double nephrectomy and tying a cannula in the bladder, seven to ten grams of dextrose per kilogram were injected intravenously in from twenty to fifty minutes, washing the bladder several times during and after the injection. The washings were then analyzed. The results are briefly as follows. In nine experiments either no sugar or else indeterminable amounts were found, and in four experiments only six to eleven milligrams, *i. e.*, 0.05 to 0.08 per cent. of the amount injected. In addition, in some of the experiments uranine, the sodium salt of fluoresceïn, was dissolved in the injection fluid. In the experiments in which twenty milligrams were introduced, no trace of uranine, a very diffusible dye, could be detected in the washings of the bladder. In four experiments 100 milligrams were introduced; in two there was not a trace and in the other two there was a barely perceptible trace.

The conclusion may therefore be drawn that the mucosa of the bladder is practically impermeable for diffusible substances present in the blood in great excess, even in the absence of the kidneys, the chief organs of elimination of the body.

25 (721)

Pancreatic transplantations in the spleen.

By **JOSEPH H. PRATT**, of Boston, and **FRED T. MURPHY**, of St. Louis.

[*From the Laboratory of the Theory and Practice of Physic, Harvard University.*]

The influence of pancreatic transplants in the spleen on the prevention of diabetes was studied. The spleen was selected because it has been shown that transplanted bits of tissue are especially well nourished in the pulp of this organ. In nine animals, bits of the pancreas of various size were buried in the

pulp of the spleen. The method used by Payr in transplanting thyroid tissue was followed. In four animals (2 dogs and 2 cats) pancreatic cells were demonstrable in the spleen. The interval between the operation and the death of the animal varied from 18 hours to 13 days. In five animals (all dogs) no pancreatic remains were found. Autolysis was rapid. In one experiment all the pancreatic cells had disappeared 21 hours after the transplantation. The bit of tissue found 13 days after the transplant measured only 1 mm. by 0.1 mm. It consisted of normal appearing acini surrounded by connective tissue. No islands of Langerhans were demonstrable.

In one dog a large pancreatic graft was placed in the spleen with the blood supply preserved by means of a mesenteric stalk. Three weeks later the original blood supply was cut off and all the pancreatic tissue except the graft extirpated. Diabetes did not develop, but the tolerance for glucose fell within a few weeks to a low point. At the autopsy 187 days after the second operation a large abscess was found in the lower part of the spleen. Projecting into the spleen from the wall of the abscess was a cone-shaped mass of fibrous tissue. In this were the remains of the pancreatic transplant measuring less than 1 cm. in length. The pancreatic tissue consisted of acini separated by connective tissue. In some of the cells, masses of zymogen granules were present. These were no demonstrable islands of Langerhans. This experiment proves that pancreatic tissue implanted in the spleen and separated from its original vascular and nervous connections can live and functionate for months.

26 (722)

The production of reversed cardiac mechanism in the dog.

By ALFRED E. COHN.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

In a series of experiments, which were published with Kessel and Mason,[1] on the excised perfused hearts of dogs, we were able to show that "excision of the sinus node results in an immedate

[1] *Heart*, June 15, 1912, iii, 311.

cessation of the contractions of the whole heart," that "after excision of the sinus node and the ensuing stoppage, the rate of the whole heart falls and does not again reach the original rate," and that "the function of the secondary pacemaker devolves on no special portion of the heart." The results of experiments performed by others and differing from ours were mentioned at that time. I have continued the experiments on dogs, anesthetized with ether, the hearts of which were left *in situ* and normally nourished. After sufficient controls were registered electrocardiographically, the sinus node was clamped with a specially constructed T clamp, 5 cm. long and 1 mm. wide. In the perfused hearts the rate fell 10 to 70 beats, usually 30 to 50 beats, after the node was excised. In the present series the fall in rate was 10 to 90, usually about 50. Stoppage, which is a usual phenomenon in excised hearts, occurred also in these, but very rarely. The site of the secondary pacemaker varied in these, as it did in the perfused hearts. Photographs of three hearts showing the areas of the auricular surfaces which had been included in the jaws of the clamp, and a series of curves from each of the corresponding experiments and of one in addition were demonstrated. The relation of the disturbed function of the heart to the area clamped will be investigated histologically and reported in detail later. The curves indicate that in each of these cases, there was a dislocation of the pacemaking function from the site responsible for it, and that, after clamping had taken place, this function devolved upon the ventricles, the junctional tissues or another portion of the auricle. In one of the experiments, first the ventricles (more probably the junctional tissues) and later the auricles set the pace. One of the experiments yielded curves which bore a close resemblance to those obtained from a patient by Williams and James,[1] in which the wave representing auricular contraction was found between the R and T waves, and was inverted. It is demonstrated by the experiments that after clamping an area along the sulcus terminalis, a site other than the normal pacemaker assumes the pacemaking function and that a "reversal of the cardiac mechanism" takes place.

[1] PROC. SOC. EXP. BIOL. AND MED., Oct. 16, 1912, X, 13.

27 (723)

Distribution of amino acids in the body.

By DONALD D. VAN SLYKE and GUSTAVE M. MEYER.

[*From the Rockefeller Institute for Medical Research.*]

In a recent paper (*Journ. Biol. Chem.*, Sept., 1912) we have shown by direct determinations (nitrous acid method) of the amino acid nitrogen of the blood, that during the digestion of protein amino acids pass from the alimentary canal into the blood. The amino acid nitrogen reaches only a relatively low concentration in the blood (3–5 mg. per 100 c.c. after 24 hours fast, 10–12 mg. during digestion), because, as was found after intravenous injection of alanine, the amino acids are either destroyed or removed with great rapidity by the tissues. The object of the present work was to obtain direct proof, by analysis of the tissues themselves with the nitrous acid method, as to whether the amino acids disappear from the blood as the result of destruction, of synthesis to more complex compounds, or of mere concentration in the tissues. The last explanation is the correct one. The tissues (muscle, liver, kidney, spleen, pancreas) contain, stated very roughly, about ten times the amount of amino acid nitrogen, per 100 grams, that is found in the blood. The amount varies in the different tissues, the brain being especially low, but about the same order of magnitude in this respect appears to be normally maintained among the different organs. Amino acids injected into the blood, or absorbed during digestion, are immediately taken up by the tissues; but the removal, although rapid, is never complete, equilibrium being reached and maintained between blood and tissues respectively. The means by which the amino acids are held in the tissues appear to be physical rather than chemical; for the acids can be extracted by even cold water or dilute alcohol. The fact that the amino acids are many times more concentrated in the tissues than in the blood excludes osmosis as an explanation. The facts thus far known are consistent with the assumption that the amino acids are taken up from the blood and held in the tissues by adsorption, chemical transformation of the adsorbed amino acids following later. This transformation occurs much more rapidly in the liver than in the muscles.

28 (724)

The fate of parenterally introduced glycogen in human and experimental diabetes.

By ROBERT A. COOKE.

[*From the Laboratory of Chemical Pathology, Cornell University Medical School.*]

It has been shown by F. Voit and P. Mayer that glycogen parenterally introduced into the normal body of man or lower animal, is utilized, there being no elimination of glycogen, dextrin or glucose in the urine. Mendel repeated this work with rabbits, cats and dogs and found, on the contrary, that 5 per cent. to 18 per cent. of the glycogen introduced was eliminated as a dextrin over a period of two days.

In human and experimental pancreas diabetes the administration of glycogen gave the following results:

I. Human diabetes, first trial, E. P., age 16 yrs., wgt. 110 lbs.
   Glycogen subcutaneously, 28.0 gm.
   Glycogen in urine as gluc. 28.0 gm.
   Elimination time, under 24 hours.

II. Human diabetes, second trial (same case).
   Glycogen subcutaneously, 21.5 gm.
   Glycogen in urine as gluc. 21.5 gm.
   Elimination time under 24 hours.

III. Complete pancreas extirpation; bitch, 8.5 kilos.
   Glycogen intraperitoneally, 9.5 gm.
   Glycogen recovered in urine as glucose 6.0 gm. = 66 per cent.
   Glycogen recovered in urine as dextrin 3.5 gm. = 34 per cent.
   Elimination time under 24 hours.

IV. Complete pancreas extirpation; bitch, 14.5 kilos.
   Glycogen intraperitoneally, 25 gm.
   Glycogen recovered in urine as glucose, 22.0 gm. = 88 per cent.
   Glycogen recovered in urine as dextrin, 3.0 gm. = 12 per cent.
   Elimination time under 22 hours.

These observations show definitely that glycogen is not a utilizable form of carbohydrate for the diabetic organism and they indicate that there is a more rapid conversion of glycogen to glucose in the diabetic than in the normal body.

29 (725)

Decerebration and the action of morphine in frogs.

By J. S. GITHENS.

[*From the Laboratory of Pharmacology and Physiology, Rockefeller Institute.*]

Morphin given in sufficient dose to normal frogs causes tetanus which does not come on, however, until several hours or even days after the injection.

I have found that decerebration hastens the onset of tetanus and also causes a marked reduction in the amount of morphin required to induce tetanus.

The smallest dose with which tetanus can be induced regularly in normal frogs at room temperature is ⅓ of a milligram per gm. (10 milligrams for a 30 gm. frog). This tetanus comes on in about 24 hours.

In decerebrated frogs, at room temperature, tetanus comes on after such a dose in from ½ to 6 hours, and may be induced with certainty after 6 to 24 hours by a dose of 1/10 milligram per gram (3 mg. for a 30 gram frog).

When frogs are kept cold tetanus can be induced by much smaller doses, as we have stated in an earlier paper. Thus, intact frogs kept in the cold show tetanus after doses of 1/30 mg. per gm. (1 milligram for a 30 gram frog). Tetanus comes on after such a dose in from 18 to 24 hours. Decerebrated frogs show tetanus after such a dose in from 4 to 12 hours, and it may be induced with certainty by doses of 1/300 milligram per gm. (1/10 mg. for a 30 gm. frog) after an interval of 1 to 3 days.

Frogs with the entire brain including the medulla destroyed, do not respond as well as frogs with the entire brain except the medulla destroyed.

30 (726)

Reversal of the cardiac mechanism. An additional note.

By HORATIO B. WILLIAMS and HENRY JAMES.

[*From the Physiological Laboratory of Columbia University, New York.*]

Since making our previous report on this subject, electrocardiograms have been obtained from the same patient in which occasionally the small downward deflection between R and T fails. The form of the ventricular electrogram in these instances is precisely similar to the others with the sole exception of the absence of the small downward deflection. This we regard as confirmatory evidence of the correctness of the view that the downward deflection is caused by the action of the auricles and that the usual curve is not simply an unusual ventricular complex. In the occasional instances cited conduction seems to have failed.

When this case first came under observation it seemed desirable to attempt to reproduce the condition experimentally. We have performed up to the present time seven experiments with one positive result. Rothberger and Winterberg have published curves showing transient reversal resulting from simultaneous depression of the sinus-region of the right auricle with cold and stimulation of the left ganglion stellatum. The apparent permanence of the condition in our patient leads us to think rather of an anatomical lesion than of nervous influences as the cause of the condition. Our experiments have consisted in depression (with anode) crushing, and excision of the region of the sinus node. In some instances nearly the entire anterior wall of the right auricle including a large part of the walls of the venæ cavæ where they fuse with the auricle, have been excised.

Slowing of the heart beat was the usual result and the auricles and ventricles generally beat nearly or quite simultaneously. In the single positive experiment auricular fibrillation resulted from the application of a clamp to the upper part of the sinus region where the greater bulk of the node is usually found. This persisted for twenty minutes and stopped immediately on application of a second clamp immediately below the first. The auricles

and ventricles then beat simultaneously. The application of a third clamp at the junction of the inferior vena cava and auricle was followed by reversal of the mechanism which persisted until the animal was killed.

The occurrence of reversal but once in a series of seven experiments has occasioned no surprise. We had surmised that after destruction of the usual pacemaker it would be quite fortuitous should the next most irritable focus lie below the auricles.

Before definite conclusions can be drawn regarding any relation which may be thought to exist between the clinical and experimental conditions it will be necessary to determine whether this is the only lesion which can give rise to a permanent reversal of the cardiac mechanism.

31 (727)

The intercalated discs of atrophied heart muscle.

By H. E. JORDAN.

[*From the Anatomical Laboratory, University of Virginia.*]

In two earlier papers[1] I presented evidence in support of my interpretation of intercalated discs as irreversible contraction bands. In a more recent paper the idea was tested by appearances in a natural experiment, namely, extremely hypertrophied heart muscle.[2] The conditions here obtaining were in perfect accord with, and confirmed the plausibility of, my previous interpretation. Since then I have had opportunity to study lesser degrees of hypertrophy, as well as an excellent specimen of atrophy[3] (weight of heart 180 grams). It is the purpose of this note to complete my report of observations on intercalated discs by a record of my findings in atrophied heart muscle, and to reëmphasize the point that all the evidence, including ontogenetic, com-

[1] Jordan, H. E., (1911) "The Structure of Heart Muscle of the Humming Bird, with Special Reference to the Intercalated Discs," *Anat. Rec.*, 5: 11; (1912; with Mr. K. B. Steele) "A Comparative Microscopic Study of the Intercalated Discs of Vertebrate Heart Muscle," *Am. Journ. Anat.*, 13: 2.

[2] Jordan, H. E. (1912), "The Intercalated Discs of Hypertrophied Heart Muscle," *Anat. Rec.*, 6: 9.

[3] For this material I am indebted to Dr. W. H. F. Addison, of the University of Pennsylvania.

parative anatomical and pathological (experimental—including the phenomena of fragmentation and segmentation) data points to the same conclusion, namely, that the so-called "discs" are aggregations of irreversible contraction foci on the myofibrillæ in the form of bands variously modified by a variety of normal mechanical and pathological, both chemical and mechanical, factors.

The first step, and central fact, in the chain of observations leading to the above conclusion respecting the origin and nature of the discs is the close similarity, amounting practically to an identity, between the normal contraction band in contracted muscle fibers and the simplest type of disc. This correspondence of appearance is very striking in the humming-bird's heart. In the relaxed fiber the *Z*-line is very conspicuous but delicate.[1] The *Q*-disc is wide and pale. In the contracted fibers the appearance is one of an alternation of robust dark (*Q*) and light (*J*) bands of approximately equal thickness. These are the same conditions that obtain in striped muscle generally in relaxed and contracted states respectively. In contraction the *Z*-line appears to become thickened by accumulation about it of dark (*Q*) substance. Of course the changes take place in the myofibrillæ. We need simply postulate local inability (due to strain, whether an instant or cumulative effect) on the part of a contraction band to reverse (or relax) to pass to the first step in the formation of a disc. The similarity between such "bands" and "discs," as also their peripheral and frequently supernuclear position, renders the assumption almost a certainty. Further support to this interpretation is derived from the following ontogenetic facts: (*a*) the discs begin to make their appearance coincidently with the cross striations; (*b*) they are at first exclusively of the simple homogeneous band type; (*c*) they increase in number (and complexity) at least to the time of full growth of the heart; (*d*) they are permanent structures throughout life. Further confirmative data are these: (*a*) step-forms are rare in birds, and absent in lower forms, the prevailing disc being of a type corresponding to the earlier ontogenetic forms in mammals; (*b*) in hypertrophied hearts the discs are exactly of a form expected from a modification of a simple

[1] Prepared according to Zimmermann's technique.

"comb-type" under the influence of longitudinal and transverse gross enlargement, and multiplication of myofibrillæ, *i. e.*, irregular zig-zag form; (*c*) mechanical ruptures in normal hearts are not localized along the discs, while in pathological fragmentation and segmentation the lines of fracture are largely limited to these levels. Of further significance is the fact that the presence of step-forms coincides with the condition of more profuse branching, hence absent in forms below birds. Step-forms would seem to be due to tension at various angles to the main longitudinal axis of the fibers.

The predominant type of disc in atrophied heart muscle is the comb-type. Fractures again occur almost exclusively at these levels. The discs in pathological heart muscle appear to be regions of weakness, probably in part at least due to chemical change. The main steps in the formation of the discs are conceived as follows: (1) irreversible contraction band (apparently homogeneous); (2) comb-discs, the length of the "teeth" varying according to the total amount, or degree, of traction on the modified regions of the included fibrils; and (3) zig-zag discs, due to a combination of the factors of longitudinal and transverse tension, and increase in number of fibrils by longitudinal splitting, *e. g.*, in hypertrophied cardiac fibers. Under the condition of general compaction which prevails in atrophied cardiac fibers, we should expect to find exactly the type of disc actually present, namely, a comb-type. Comb-discs according to the above interpretation of their origin are obviously regions of relative weakness, of relatively greater degree in pathological hearts where there is operating very probably an additional chemical factor. Hence in atrophied hearts fragmentation when present is limited to these levels. The discs once formed are permanent structures undergoing various modifications according to physiological conditions. Direct observation reveals a close similarity between the contraction band and the simplest type of disc. Hence the evidence for the origin of the various types of discs from irreversible contraction bands is practically complete.

32 (728)

The differentiation and specificity of starches in relation to genera, species, etc.: stereochemistry applied to protoplasmic processes and products, and as a strictly scientific basis for the classification of plants and animals.

By EDWARD TYSON REICHERT.

[*From the S. Weir Mitchell Laboratory of Physiology, University of Pennsylvania.*]

Under the foregoing title the results of an elaborate investigation will shortly appear as Publication No. 173 of the Carnegie Institution of Washington. This research is supplementary and complementary to, and in support of, Publication No. 116 entitled, "The Differentiation and Specificity of Corresponding Proteins and Other Vital Substances in Relation to Biological Classification and Organic Evolution: The Crystallography of Hemoglobins." A preliminary review of the latter will be found in the PROCEEDINGS of the Society, 1908, V, 66–68.

Studies of the histological, physical, physico-chemical and chemical properties of over 300 starches from various plant-sources were made. Among the most important conclusions reached are the following:

1. Starch is not a unit substance but exists in a vast number of stereoisomeric forms. Starch from any given plant and of every mature starch grain is a mixture of different forms of starch-substance.

2. Starches from different plants exhibit constant and specific characters in relation to genera, species and varieties, by which the plant can be identified.

3. The stereochemic peculiarities of starches and other complex organic metabolites constitute *a strictly scientific basis for the classification of plants and animals.*

33 (729)

The motor cortex and pyramid tract in the raccoon (Procyon lotor Linn.).

By SUTHERLAND SIMPSON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

The raccoon is a very intelligent animal and, as one would expect, relatively to the size of its body, it possesses a large brain with a highly convoluted cerebral cortex. Little appears to be known, however, about its cortical topography or the fiber tracts of its central nervous system.

The present note refers to the experimental localization of the motor areas and the subsequent tracing of the pyramid tract by the method of ablation and secondary degeneration. Five full-grown specimens were obtained. The cerebral cortex was exposed on the left side, under ether anesthesia, the motor areas were localized both by the bipolar and unipolar methods and then removed. The wound was closed and the animal allowed to live for about two weeks when the left hemisphere was exposed and explored in the same way before the animal was finally dispatched by an overdose of ether. The brain and spinal cord were removed and treated by the Marchi method.

The region from which muscular responses were obtained is relatively large and well defined. It occupies the whole free surface of what may be termed the post-cruciate convolution, extending from the mesial border of the hemisphere to a little way beyond the lateral extremity of the cruciate sulcus. Unless when the current was comparatively strong, no movements were obtained from the cortex in front of the cruciate sulcus. From the mesial border lateralwards the order of the responsive areas was as follows: anus, tail, hind limb and digits, body, fore-limb and digits, head and eyes, and face, mouth and tongue, the last curving forwards around the lateral extremity of the sulcus. The movements were readily elicited and the areas well defined but there was always some overlapping at the margins. The forearm area appeared to be the most easily excited, that is to say, it

responded to a weaker current than was necessary for the stimulation of the other areas.

The pyramid tract fibers were traced from their origin in the cerebral cortex, caudalwards, to the spinal cord. They occupied the usual positions in the crusta, pontine bundles and anterior pyramid above the decussation. In the posterior part of the medulla oblongata most of them crossed the median raphe and turned caudalwards into the lateral column of the spinal cord, but in three of the specimens examined a considerable number of fibers remained uncrossed and formed a direct ventral pyramid tract, extending along the margin of the ventral median fissure. This tract could be traced to the middle of the thoracic region where it disappeared. A few uncrossed fibers were also found in the lateral column.

It is generally believed that this uncrossed ventral tract (direct pyramidal tract) is limited to man and the anthropoid apes; such, however, is not the case, for it is present in the raccoon and is more pronounced still in the porcupine.[1]

The crossed lateral pyramid tract could be followed to the last of the sacral segments. In the raccoon this is a large tract both in relative area, in transverse sections of the spinal cord, and in the number of fibers which it contains. In no animal below the macaque monkey have I found it so extensive.

34 (730)

Note on the action of tonsillar extract.

By ISAAC OTT and JOHN C. SCOTT.

[*From the Laboratory of Physiology, Medico-Chirurgical College, Philadelphia.*]

We used the dried powdered tonsil of the calf. When a filtered infusion of two grains of the powdered tonsil was injected into the cat by the jugular vein in divided doses, it produced a great fall of blood-pressure, lasting about a minute, followed by a rise above normal, with a much slower and stronger heart beat. Increase of this dose suddenly arrested the heart. In the same animal it was also a diuretic, increasing the flow of urine twenty times the original amount.

[1] Sutherland Simpson, PROC. SOC. EXPER. BIOL. AND MED., 1912, Oct. 18, p. 15.

35 (731)

The diffusion of iodo-eosin from ether through rubber membrane into ether.

By JACOB ROSENBLOOM.

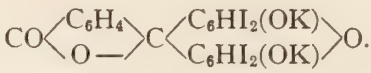
[*From the Laboratory of Biological Chemistry of the University of Pittsburgh, Pittsburgh, Pa.*]

Rosenbloom and Gies[1] have shown that many ether soluble substances diffuse from ether through rubber membrane into ether, but oddly various phospholipins do not possess this property.

Together with Boas,[2] I was able to show that various cholesterolesters diffuse from ether through rubber membrane into ether. This is very interesting on account of the high molecular weight of these esters. Cholesterol-stearate with a molecular weight of 652.51 diffuses very readily.

For some time I have been trying to find an ether soluble substance of higher molecular weight than cholesterol-stearate and which could be easily detected in the diffusate. The free dye-acid of iodoeosin fulfilled these requirements. This free dye-acid has been employed by Professor Ehrlich as a very delicate reagent for free alkali in the erythrocytes of man.[3]

Iodo-eosin is the potassium salt of tetraiodo-fluoresceïn with the following formula,



As a salt it dissolves in dilute alkalies with red color, but is insoluble in ether or any other organic solvent. The free dye-acid, however, is obtained as a yellow precipitate from the alkaline solution of iodo-eosin by adding hydrochloric acid in excess, and it dissolves readily in ether, or in any other organic solvent, but is insoluble in

[1] *Proc. Amer. Soc. Biol. Chem.*, 1910, 2, p. 8; *Jr. Biol. Chem.*, 1911, IX, p. xiv. *Biochemical Bulletin*, 1912, II, p. 64; George Crocker Special Research Fund, Vol. 3; (In press.)

[2] PROC. SOC. EXP. BIOL. AND MED., 1911, VIII, p. 132.

[3] Ehrlich-Lazarus, "Die Anemia," Vienna, 1898.

water. This free dye-acid has the following formula,

$$CO\begin{cases} C_6H_4 \\ O \end{cases}\!\!>\!C\!<\begin{matrix} C_6HI_2(OH) \\ C_6HI_2(OH) \end{matrix}\!\!>O,$$

with a molecular weight of 836.

The dye-acid of iodo-eosin may be made by dissolving ten grammes of iodo-eosin (commercial dye) in one per cent. potassium hydroxide and then adding hydrochloric acid in excess. The dye-acid is precipitated at once, it can then be filtered off and the precipitate washed with hot water till the washings are acid-free. The precipitate after drying is easily soluble in ether, forming a beautiful yellow-colored solution.

When this free dye-acid in ether solution is placed inside of an intact rubber membrane immersed in ether, it can readily be noted that in a few minutes diffusion currents are visible and the ether outside of the bag becomes colored, showing that the free dye-acid has diffused.

The bearing of our results on the question of permeability and impermeability of membranes will be considered later.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

First meeting.

San Francisco, California, December 4, 1912.

36 (732)

Chronic lead poisoning in guinea pigs.

By W. OPHÜLS.

[*From the Pathological Laboratory of Stanford University.*]

Of the twenty-eight guinea pigs treated with sublethal doses of carbonate of lead seven (25 per cent.) showed a peculiar condition to which so far attention does not seem to have been directed. There has developed a hemorrhagic, sero-fibrinous inflammation of the pericardium, of the peritoneum in the upper part of the peritoneal cavity and occasionally also of the pleuræ. In the pericardium the lesion commences with a hemorrhagic exudate followed by the formation of fibrinous deposits especially on the parietal layer and ending with organization with marked thicken-

ing and occasionally adhesions. In the pleuræ one finds as a rule a simple serous exudate without deposits but occasionally heavy deposits of fibrin also. In the peritoneum also the condition commences with fibrinous deposits and eventually leads to marked peritoneal thickening more especially in the upper part of the peritoneal cavity. The capsules of liver and spleen are much thickened and the contraction of the thickened capsule often causes marked deformity of the liver. The lesions produced in this way are closely analogous to Curschmann's "Zuckergussleber." The spleen is always more or less enlarged, but this is due to the excessive destruction of erythrocytes by the lead and is found in all animals.[1]

It was only natural to suspect the presence of a bacterial infection of the serous membranes in these cases. A very careful bacteriological examination was made in several of the cases, but it was entirely negative.

Whether the hemorrhagic exudation which ushers in the process is due to the lead anemia appears to me a question well worth debating. Smears of the exudate show few leucocytes and often many nucleated red blood corpuscles, even megaloblasts.

In one case (46) adhesions had formed in the upper part of the peritoneal cavity with partial strangulation of duodenum and intestine and extreme dilatation of the stomach.

In the liver the periportal connective tissue is moderately but distinctly thickened in some cases. One always finds small areas of fatty degeneration and necrosis of the liver tissue. In one case (46) they were quite large and show beginning liquefaction in the center (due to apparently secondary invasion of bacteria). Whether these degenerative lesions are due to circulatory disturbances produced by the shrinkage of the thickened capsule or whether they are independent of this and toxic in origin, it is difficult to decide, although in one case the lesions in the liver were well marked but those in the capsule only slight. The bearing this may have on the modern conception of cirrhosis as closely connected with certain types of anemia is evident.

[1] It is interesting to know that Charcot and Gombault found acute pericarditis in their guinea pig 4, hemorrhagic pericarditis in their guinea pig 9, subacute pericarditis in their guinea pig 15, and pericardial thickening in their guinea pigs 6 and 14 (5 out of 15).

It is interesting to observe in these animals a condition so entirely analogous to what has been described in man as polyserositis, or a polyorrhomenitis by Italian investigators, of which Curschmann's Zuckergussleber and Pick's pericarditic pseudocirrhosis are only special manifestations. The relations of these conditions in man to chronic lead poisoning and to chronic anemia remains to be studied.

37 (733)

Chronic lead poisoning in guinea pigs. Its relation to chronic nephritis.

By W. OPHÜLS.

[*From the Pathological Laboratory of Stanford University.*]

During the last years I have been repeating the experiments of Charcot and Gombault[1] on chronic lead poisoning in guinea pigs. Twenty-eight guinea pigs were given sublethal doses of carbonate of lead for periods ranging from one month to three years and ten months. Fourteen of these experiments lasted over one year.

The lesions in the kidneys were much less striking than one would expect from the report of Charcot and Gombault. In all cases there was a limited necrosis and desquamation of the epithelium with marked evidences of regeneration especially in the ascending limbs of the loops of Henle. In some cases the epithelium in places was heavily pigmented. Occasionally there were seen a few glomeruli with slightly thickened capsules. There were only two of the twenty-eight experiments in which more advanced lesions were discovered in the kidneys. In one case (69) in which the guinea pig had received over thirty grams of carbonate of lead in three years the kidneys were actually granular and the cortex distinctly narrow. The lesions in this case consisted in collapse of tubules over large areas with marked development of fibrous tissue between them. There were many casts. The glomeruli showed marked fibrous thickening of the capsules and cystic dilatation. The other guinea pig (51) which received about four grams of carbonate of lead in twenty months showed similar con-

[1] Charcot et Gombault, "Note relative à l'étude anatomique de la nephrite saturnine expérimentale," *Arch. de Phys.*, 1881, 2 s., VIII, 126.

ditions but they were not so well marked. Calcareous infarcts were not found in any of the kidneys. No vascular lesions were ever found in the aorta or in the branches of the renal arteries. On the whole the condition produced in these few animals does not resemble human nephritis, but is much more similar to the lesions observed in experimental uranium nephritis.

38 (734)

Agglutination of encapsulated bacteria.

By J. G. FITZGERALD.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

During the past year a systematic study of the group of encapsulated bacteria (including *Bacterium pneumoniæ* Friedländer, *Bacterium rhinoscleromatis* v. Frisch, *Bacterium ozenæ* Abel-Löwenberg, and *Bacterium capsulatus mucosus* Fasching) has been carried on, employing for the purpose biometrical methods somewhat similar in character to those suggested by Winslow[1] in his work on the Coccaceæ.

During the course of the investigation, immunological methods have been used. At the outset, the reaction of agglutination was tried. Paltauf was the first (quoted by Beham[2]) to suggest that the agglutination of encapsulated micro-organisms is inhibited because the bacilli are surrounded by a slimy nucleo-protein capsule. Porges[3] was able to supply experimental proof of this. v. Eisler and Porges[4] then elaborated a method of removing the capsule, after the application of which these bacteria were agglutinable.

My own work done independently of Beham has given results in harmony with his. I have found that *Bacterium rhinoscleromatis* on injection into rabbits yields a potent agglutinating serum.

Using this serum, agglutination not only of the homologous microörganism has been obtained but a positive result was found

[1] Winslow, "Systematic Relationships of the Coccaceæ," John Wiley & Sons, New York, 1908.

[2] Beham, *Central. f. Bakt.*, Abt. 1, Orig., Bd. 66, Heft 1, p. 110.

[3] Porges, *Wiener klinische Wochenschrift*, 1905, No. 26.

[4] v. Eisler and Porges, *Central. f. Bakt.*, Abt. 1, Orig., Bd. 42, Heft 7, 660.

to occur also when four other strains of the same species, from widely different sources, were tested. The bacteria agglutinated in a dilution of the serum, ranging from 1–400 to 1–800. Two strains of *Bacterium ozænæ*, one strain of *Bacterium capsulatus mucosus* Fasching, and one strain of *Bacterium pneumoniæ* Friedlander, were not agglutinated at all by the same serum. The strains of *Bacterium rhinoscleromatis* which agglutinated were no longer encapsulated. All the other species still showed a capsule. One of the strains of rhinoscleromatis when originally isolated was not agglutinated by the serum of the patient from whom it was isolated and at that time it was encapsulated (Thro, *Proceedings New York Pathological Society*, April and May, 1910). This microörganism, then, was not agglutinable when encapsulated shortly after isolation. Later, it lost its capsule and became agglutinable. As far as my work has gone, I have found that of the species here considered rhinoscleromatis most easily loses its capsule when grown on ordinary laboratory media and becomes agglutinable.

Beham has shown that encapsulated as well as non-encapsulated species of this group have antigenic properties and may produce agglutinins. I have no data on this point as yet. The acid agglutination reaction of Michaelis, as modified by Beniasch[1] did not agglutinate *Bacterium rhinoscleromatis* without a capsule, or *Bacterium capsulatus mucosus* possessing a capsule.

Bacteria of the species here considered are not agglutinable when encapsulated, but may become so when they lose their capsule in the course of months of growth on laboratory media. Having lost their capsules, these species may be differentiated by the reaction of agglutination. Thus *Bacterium rhinoscleromatis* has been shown to differ from *Bacterium ozænæ* and from *Bacterium pneumoniæ* Friedlander, in that it more easily loses its capsule and is then agglutinated by its specific serum.

[1] Beniasch, *Zeitschrift für Immunitatsforschung*, Vol. XII, Heft 3, 1912, 268.

39 (735)

Relative frequency of B. coli communior in contaminated water.

By J. G. FITZGERALD.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

Some recent work of my students has been interesting in connection with the relative frequency of occurrence of the species of the *B. coli* group in water contaminated with human feces. The *B. coli* group consists of four species: *B. communior* (Durham), *B. communis* (Escherich), *B. aerogenes* (Escherich), *B. acidi lactici* (Hueppe). The general characteristics common to all species are: short, Gram-negative, cocco-bacilli, with rounded ends, non-spore bearing, fermentation of dextrose, glucose and lactose with gas production, non-liquefaction of gelatin after 14 days, indol production, and reduction of nitrates to nitrites. In general, also, litmus milk is acidified and coagulated. Further differentiation of the members of the group is possible by means of fermentation tests in the sugars dulcite, saccharose, mannite and raffinose. *B. communior* and *B. communis* ferment dulcite; *B. aerogenes* and *acidi lactici* do not. *B. communior* and *B. aerogenes* ferment saccharose. *B. communis* and *B. acidi lactici* do not. MacConkey[1] isolated a large number of strains of these species from feces and found that their relative frequency was as follows:

	Per Cent.
B. communior	23
B. communis	37
B. aerogenes	15
B. acidi lactici	25

Graham Smith[2] in the analysis of thirty-five strains isolated from flies found, on the other hand, a predominance of *communior* (43 per cent.), *communis* occurring much less frequently (17 per cent.). *B. aerogenes* (11 per cent.) and *B. acidi lactici* (29 per cent.) did not differ greatly in their occurrence.

Winslow and Walker[3] in twenty-five strains isolated from

[1] MacConkey, *Journal Hygiene*, 1905, Vol. 5, p. 333.

[2] Graham-Smith, Report of Local Government Board, London, 1909.

[3] Winslow and Walker, *Science*, 1907, Vol. 26, p. 675.

feces found 28 per cent. of *communior*, 60 per cent. *communis*, 4 per cent. *aerogenes*, and 8 per cent. *acidi lactici*.

The results of an analysis of thirty-two strains isolated in my laboratory from human feces were as follows:

	Per Cent.
B. coli communior	65
B. coli communis	28
B. aerogenes	3.5
B. acidi lactici	3.5

The saccharose fermenting species, then, may predominate in water recently contaminated with human feces.

40 (736)

Production of creatinine by bacteria.

By J. G. FITZGERALD and CARL L. A. SCHMIDT.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California, Berkeley, California.*]

Very little work has been done to determine whether creatinine is produced by bacteria growing on media free from creatin and creatinine. Germán,[1] in a brief review of the literature on this subject, makes mention of only three contributions. In his paper, Germán gives the result of an investigation of thirty-five species of bacteria to determine whether they were able to produce creatinine. In summing up, he says that this characteristic might be of value in the differentiation of closely allied species.

Our work was undertaken to ascertain: (1) Whether creatinine production is of any value in the differentiation of groups of closely allied species; (2) to determine the method best suited for studying creatinine production by bacteria; (3) to ascertain what amounts of creatinine were produced so that certain quantitative studies might be undertaken.

A medium composed of 2 per cent. Witte's peptone and .5 per cent. salt furnishes a creatin and creatinine free medium. Twenty-eight strains of bacteria, belonging to the mucosus capsulatus group of microörganisms, including *B. rhinoscleromatis*, *B. ozænæ* and *B. lactis aerogenes*, were grown on the above medium for eight

[1] Germán, *Centralbl. f. Bakt.*, I. Abt. Orig., Bd. 63, Heft 7, 1 June, 1912.

days. Ten cubic centimeters were then tested for creatinine by Weyl's method (adding several c.c. of a 10 per cent. NaOH solution and several drops of a freshly prepared sodium nitroprusside solution, a positive reaction being indicated by the immediate appearance of a dark red zone which soon turns to a greenish color) and 50 c.c. were tested by the Folin method (adding 7.5 c.c. saturated picric acid solution and 2.5 c.c. sodium hydrate, a positive reaction being indicated by the production of an orange-red color similar to the color produced by a potassium bichromate solution). As controls, a strain of each of *B. coli communis*, *B. proteus*, *V. choleræ asiatica*, *M. aureus*, *M. albus*, and *V. Metchnikovii* were examined at the same time. A sterile peptone solution itself gives with the Folin method a slight color which to a certain extent interferes with the test; this, however, can be eliminated by comparing the solution to be tested with the control. In only one case, that of *B. proteus*, was the Folin reaction decidedly positive, while *V. choleræ asiatica*, and several others gave a somewhat doubtful positive result. With the Weyl reaction a much more accurate determination of the presence of creatinine can be made.

As regards the *Mucosus capsulatus* group, we found that creatinine production is no criterion in differentiating the species, since nearly all the strains gave negative reactions. That the amount of creatinine which is produced as a result of bacterial metabolism is very small is indicated in the results obtained by the Folin method. That certain bacteria produce creatinine more readily and in larger amounts than others is shown by the more strongly positive reactions given by *B. proteus*. Germán's results tend to show the same thing. His inability to get a positive creatinine reaction with certain organisms in twenty-four hours, which after a longer period of time gave positive reactions, does not show that no creatinine was produced during the first twenty-four hours, but rather that the amounts produced were so small that the test appeared to be negative. That there are certain bacteria which either do not produce any creatinine when grown on a peptone medium or produce amounts of creatinine so small that the Weyl reaction appears negative is shown both by Germán's and our results.

We were unable in our work to parallel Germán's results in the case of all the species which he claims produce creatinine. This we believe to be due to the fact that the ability to produce creatinine is a characteristic which may easily be lost by bacteria just as is the power of producing indol.

41 (737)

Regeneration of bone from periosteum.

By S. L. HAAS.

[*From the Pathological Laboratory of Stanford University.*]

In an endeavor to establish the factor that periosteum plays in the regeneration of bone, the following experiments were performed on the ribs of rabbits.

Several experiments consisting merely of a subperiosteal resection of the rib showed after 20 days a cartilaginous-like material filling the entire space; while after a longer time complete filling in with bone.

The next endeavor was to isolate the periosteum so as to prevent bony elements from growing in to the periosteal space.

In one experiment the rib ends were capped with lead—after 12 days no evidence of regeneration. This method was discarded as it was thought better to try and raise the rib from its periosteal covering without severing the ends, and isolate it by sewing muscle beneath the raised rib.

In 10 experiments of this nature the results uniformly showed a tendency for bone to grow in at the angle where the rib was raised from its periosteal bed. In one experiment after 59 days the whole area was filled in by bone, although in another after 8 months there was only a small spicule of bone growing in at the sternal side. In only one experiment after 26 days was an isolated island of bone found, free from connection with bone elements.

Although this does not prove the point sought, it at least emphasizes the tendency of bone to grow in the direction of existing periosteum.

At this stage it was noticed that there was a difference at times in bone regeneration and it was suggested to try the effect

of blood clot. The experiment was performed in the same way as the previous one except that blood clot was placed in the periosteal gutter left by the raised rib. After periods of 48, 44, 39, 23, and even 14 days, the whole area was found to be occupied by a calcareous material. Two ribs in the same animal were similarly treated with the exception that in one no blood clot was used. The one containing blood clot showed considerably more bone formation. This showed the marked increase of bone in the presence of blood clot.

In order to rule out ingrowth of bone from the side, the following method was resorted to. Extensive resections of a rib from near the vertebral junction, to and including about 1 cm. of costal cartilage. Hot paraffin was poured in at the vertebral and sternal sides, allowing a free gap between. This gap was filled with blood clot and all sutured in place. In four experiments of this nature there always was found calcareous material, separated grossly from any bony tissue. A few experiments without blood clot were used as controls to the last. These showed some calcareous material. In one particular instance considerable bleeding was noticed at the end of operation, and in this case a heavy bony deposit occurred. Thus the factor of post-operative hemorrhage might explain discrepancies of results of these experiments and those of other experimenters.

An interesting point was noticed in cases where costal cartilage was dissected away, in that there was an especially marked tendency for regeneration of bone at costal cartilaginous junction. If we consider this junction as an epiphysis it is remarkable that we should get so much regeneration after its destruction, evidently from the periosteum.

In these experiments the periosteum has been considered as one usually leaves it after surgical removal of bone. In conclusion it appears that periosteum under certain conditions has the power of regenerating bone, especially in the presence of blood clot. Also, that it always exerts a stimulating effect on bone formation.

42 (738)

Preliminary report on the influence of lecithin and cholesterin upon the growth of tumors.

By T. BRAILSFORD ROBERTSON and THEO. C. BURNETT.

From the Rudolph Spreckels Physiological Laboratory of the University of California.]

Sixty-one white rats, obtained from local sources and hereafter designated "Local" were inoculated in the axillary region with portions of a Flexner-Jobling carcinoma which was obtained from the Rockefeller Institute for Medical Research through the kindness of Dr. Peyton Rous. The number of successful inoculations, determined after 19 days, was 42, or 69 per cent.

Sixty-four rats obtained from dealers in Chicago and hereafter designated "Chicago" were similarly inoculated with portions of a Flexner-Jobling carcinoma which had been obtained from the Rockefeller Institute for Medical Research early in the year and propagated through four generations of rats in this laboratory. The number of successful inoculations, determined after 18 days, was 55, or 86 per cent.

Both sets of tumors grew rapidly, attaining average diameters of 15.0 and 11.9 millimeters after 19 and 18 days respectively.

Beginning upon the 19th day in the case of the "Local" and upon the 18th day in the case of the "Chicago" animals, each of the two groups of animals was sorted without selection into three batches, of which one (consisting of 12 animals in each case) served as controls, another (10 "Local" and 13 "Chicago") received 1 c.c. of a 3.9 per cent. suspension of cholesterin in N/10 sodium oleate solution, which was injected hypodermically directly into the tumors every 2d or 3d day (three times a week); the third batch (20 "Local" and 30 "Chicago") received 1 c.c. of a 2 per cent. aqueous emulsion of lecithin similarly injected into the tumors upon the same days.

It was found that in the pre-metastatic stage cholesterin causes a very notable acceleration of the primary growth, the gain in diameter between the 19th (18th) and 24th (23d) days being 11.6 mm. in the "Local" and 11.4 mm. in the "Chicago" animals as

compared with 4.8 mm. and 2.9 mm. respectively in the controls. The acceleration of the growth of the primary tumor by cholesterin is not very evident in the metastatic stage (31st to 38th days), but the tendency to form metastases and the rate of metastatic growth are very markedly increased.

Lecithin, on the contrary, diminishes the tendency to form metastases, retards metastatic growth when it does occur and, in some instances (the "Local" animals) also retards the growth of the primary tumor in the post-metastatic period.

43 (739)

Note on the cock's comb test for the activity of ergot.

By ALBERT C. CRAWFORD and JAMES P. CRAWFORD.

[*Laboratory of Pharmacology, Leland Stanford Junior University.*]

The cock's comb test has become a popular method of determining the activity of ergot preparations. It was introduced because gangrene frequently occurred in epidemics of ergot poisoning, and because bluing of the cock's comb was believed to be due to arterial constriction. This view was supported by von Recklinghausen's interpretation of the microscopical examination of the comb in chronic ergot poisoning of cocks. We were impressed by the fact that in ergot poisoning often the only pathological feature was venous dilatation and we believe that venous dilatation is probably the real cause of the bluing of the comb. It is admitted that the intravenous injection of epinephrin causes a rise in blood pressure, mainly from vaso-constriction and we have found that it will blue the cock's comb, but the bluing only comes on as the blood pressure falls and persists for an hour or two; in other words it occurs at a time when arterial-constriction has subsided. Large doses of paraldehyde given per os, or the inhalation of amyl nitrite, will also cause bluing of the comb.

On subcutaneous injection neither adrenalin nor p. oxyphenylethylamine caused this bluing. Dale claims that much of the activity of ergot preparations is due to p. oxyphenylethylamine. Now while the subcutaneous injection of 25 mg. of p. oxyphenylethylamine caused marked symptoms in cocks, there was no bluing, hence one would argue that the subcutaneous injection

of ergot into cocks cannot be considered a quantitative method for testing ergot. Oncometric studies of the cock's comb during acute ergot poisoning are now being made.

44 (740)

An experimental study of poison oak.

By Edward von Adelung.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

Experiments show that the toxic principle of *Rhus diversiloba* while not volatile can poison at a distance by means of mechanical carriers. It is not destroyed by subjection for one hour to 100° C. and is carried, potent, by the smoke from burning *Rhus* plants. The dermatitis produced by this plant is a purely local affection and is not spread by the blood or lymph or by the serum of the blebs. The reaction of the sweat has no relation to individual susceptibility.

Absolute immunity in man has been claimed but was not found to exist, on repeated attempts, in any of the individuals tried (6 persons). In spite of the work of Ford, the conclusion is arrived at that experimental immunity in animals to *Rhus* toxin has not been proved. The author, working with pure toxin (glucoside) produced by the method of Syme, was unable to intoxicate animals with any reasonable amount. Ford's work was done with a commercial fluid extract containing various impurities. A permanent aqueous suspension of the alcoholic solution of the pure toxin can be prepared, and remains toxic for the human skin. As much as 0.025 gm. of this preparation of toxin can be given intravenously to a 2,000-gram rabbit without fatal effect, and as much as 0.03875 gm. can be given to a 280-gm. guinea pig subcutaneously without fatal effect. Pure alcoholic extract when given subcutaneously produces severe necrosis and death owing to the alcohol itself used as a menstruum, but with no lesions attributable to the toxin. No skin lesions were produced in monkeys, rabbits, or guinea pigs as a rule, but a slight dermatitis was produced on the rabbit's ear at times.

Inasmuch as animals can not be killed by the pure toxin, it is impossible to demonstrate antitoxic effect in the serum of animals that have received repeated doses of the toxin. The Bordet-Gengou fixation reaction failed to demonstrate the presence of antibodies in the sera of animals so treated.

The simplest prophylactic measure against *Rhus* poisoning is to wash well with soap and hot water as soon after exposure as possible. The protection of the skin by anointing with cotton-seed oil before exposure and washing this off within a few hours with soap and water, renders prophylaxis fairly certain. The following remedies have therapeutic value: hot water, ichthyol collodion, permanganate of potassium, magnesium sulfate, and tincture of iodine.

45 (741)

The failure of union between antigen and precipitin when present in the same serum.

By HANS ZINSSER and STEWART YOUNG.

[*Stanford University.*]

It has been frequently observed that the serum of rabbits, immunized with foreign protein, may, at certain times, contain not only precipitin, but also unaltered antigen remnants. Such sera not only precipitate the antigen, but also give precipitates when mixed with other antisera prepared with the same antigen (Linosier et Lemoine, Eisenberg, Michaelis, and Fleischmann, Ascoli, Von Dungern). They have been recently studied by Gay and Rusk. It has been difficult to explain why such sera do not spontaneously precipitate since both reacting factors are present.

In the cases of two sera recently studied by us the phenomena observed were as follows: Sera "3" and "4," obtained by injecting two rabbits with horse serum on three successive days and bleeding eight days after the last injection, were perfectly clear and showed no spontaneous precipitation on standing several days. Serum "3" precipitated horse serum in dilutions of 1 to 1,000, and serum "4" in dilutions of 1 to 500. When mixed with equal quantities of an antihorse serum precipitin, which contained no antigen, both sera were precipitated, "3" more strongly than "4." Neither of

these sera fixed complement. When "3" and "4" were mixed, slow and slight, but distinct precipitates occurred, unmistakable after 12 hours in the ice chest.

On standing for one month in the ice chest sera "3" and "4" were found to have spontaneously precipitated. The precipitate showed slight complement fixation and the supernatant fluid was found on titration to have lost about one half of its precipitating power.

Such spontaneous precipitation of precipitin sera has been repeatedly observed after prolonged conservation.

Von Dungern who has done much work on this question has assumed a multiplicity of antigens and precipitins to account for this failure of union between antibody and antigen in such sera. He says "Neben dem gebildeten Praezipitin bleibt aber ein anderer Theil der Praezipitablen Substanz der keine Affinitaet zu dem gebildeten Praezipitin besitzt, bestehen, solange bis ein anderes Partial Praezipitin von den Kaninchen geliefert wird, welches sich mit Gruppen der in Loesung gebliebenen Eiweisskoerper vereinigen kann."

We do not believe that this explanation is tenable in our cases since eventual precipitation, therefore union, took place after prolonged standing.

Another explanation (Eisenberg) accounts for the phenomenon by assuming that antigen and precipitin unite according to the laws of mass action, establishing an equilibrium, in which ununited portions of each of the reacting factors are therefore found. This explanation seems incompatible with the observation that the two sera did not bind complement, pointing to the absence of united antigen and precipitin in the sera.

We believe that the failure of union of the two elements may be regarded as closely analogous to phenomena occurring under the influence of protective colloids.

This is compatible with the original failure of the two substances to unite; with their precipitation by other sera in which there is present one or the other of the reacting bodies; and with their final spontaneous precipitation on prolonged standing.

It is more difficult to account, on this basis, for the mutual precipitation of these sera which occurs when they are mixed.

For this we have sought analogy. Fresh dog serum precipitates colloidal trisulphide of arsenic. Heated dog serum precipitates the arsenic only when small quantities of the serum are added. Larger quantities again disperse the precipitate and then protect the colloidal metal from precipitation by subsequently added fresh serum.

The addition of further arsenic trisulphide to such protected mixture will disturb the balance and lead to precipitation.

It should be possible to make up two clear solutions, each containing the three bodies, but in entirely different proportions, such that upon mixing the two, the protective action of the heated serum is so reduced that precipitation will occur. Slight, slowly appearing precipitates have been produced in this way, but the results thus far obtained have merely sufficed to emphasize the likelihood of the explanation we offer. The protective action of the heated serum is so enormous that it requires a very accurate adjustment of relative amounts to ascertain the exact limit of protection.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-second meeting.

Physiological Laboratory, College of Physicians and Surgeons, February 19, 1913. President Ewing in the chair.

46 (742)

Further light on the conjugation of Paramecium.

By **Gary N. Calkins.**

[*From the Department of Zoology, Columbia University.*]

These experiments were undertaken to test the variability after conjugation of *Paramecium caudatum* in respect to the power to conjugate. An individual immediately after separating from conjugation possesses a new fertilization micronucleus and the old macronucleus. The new micronucleus divides into two, these two into four, the four into eight. During these three divisions the old macronucleus goes to pieces and the fragments are gradually absorbed in the protoplasm. Four of the eight new nuclei then metamorphose into four macronuclei, and then for the first time after conjugation, the cell divides. The progeny have two macro- and two micronuclei each. These cells immediately divide again without further nuclear division, forming four cells, each with one macronucleus and one micronucleus. The normal condition of *Paramecium* is then attained. The problem to be solved by experiment was: Do these four cells give rise to progeny which vary in respect to the power to conjugate? In order to get as many chances as possible the products of the first three divisions of each of the four cells were kept isolated, thus giving eight lines of cells from each of the original four. These sets of eight, thirty-two in all, I shall speak of as the 1st, 2d, etc., quadrants. The thirty-two lines were kept isolated in vials and fed at intervals of from six to eight days under conditions prohibitive of conjuga-

tion, from the 27th of last July to the present. From the outset marked differences in the size of the progeny of different lines were noted; the statistical proof, however, is not yet ready for presentation. A marked difference in division rate was also noted; for example, the daily division rate of representative individuals of all the lines was carefully kept for a period of 30 days in October and November. The average rate for individuals of the first quadrant was 0.65 divisions per day; of the 2d, 0.91 per day; of the 3d, 0.81, and of the 4th, 0.95 per day, a difference of from fifteen to twenty per cent. between the rates of individuals from the 1st quadrant and those of the other three. The weaker vitality of progeny of the 1st quadrant is also shown by the extinction by death of all the progeny of four lines of the eight, whereas only one line died out from the progeny of quadrants 2 and 4, and none from quadrant 3. (In quadrant 2, two lines failed to start by reason of a pathological division in the third generation.) The power to conjugate is correlated with these variations in vitality. Conjugation tests (too complex to be described here) were started in October and November, and many pairs were found in two of the four remaining lines of the 1st quadrant, none in the others. The December tests gave similar results; three of the four lines of the first quadrant giving many pairs while none were found in the other twenty lines. Again in January and February all four lines of the first quadrant furnished many pairs, but not a single pair was found in the other lines subjected to exactly the same treatment. A specific example will show how extensive the observations were. In the January test, for example, not less than 150,000 *Paramecium* belonging to the non-conjugating lines passed under my eyes, not a single conjugating pair being seen. Of the four conjugating lines not less than 50,000 *Paramecium* were observed, of which more than 2,000 pairs were in conjugation. It is evident therefore that a very decided difference exists in the total progeny of an ex-conjugant; some are potential germ-cells, others apparently are not. These experiments may give a clue to the divergent results obtained by Maupas, Woodruff and myself, which cannot be harmonized on the ground of abnormal conditions or bacterial poisons in one case and not in another. The divergence must be due to some more deeply lying cause in

the organisms themselves. The race that I worked with in 1901 was a conjugating race which died out in the 742d generation. Woodruff's long line of over 3,500 generations is a non-conjugating race and the two races cannot be compared in regard to vitality, since normal conjugation was prevented in the conjugating race, whereas in the non-conjugating race there has been no artificial prevention of a normal process. The following conclusions may be drawn; they must be considered provisional, however, since the experiments are not yet concluded.

1. The traditional view that each *Paramecium* is a potential germ cell is not true.

2. Some descendants of an ex-conjugant are potential germ cells, others are not.

3. The life history of conjugating lines has shown that if conjugation is prevented, the race dies out.

4. Weismann's hypothesis that natural death is absent in protozoa is not borne out by the facts.

5. Care must be exercised in arguing that one effect of conjugation is to bring about variations because of amphimixis until we know that such variations are not brought about by every individual ex-conjugant in its normal development.

47 (743)

Note on the intraperitoneal lysis of tubercle bacilli.

By WILFRED H. MANWARING and J. BRONFENBRENNER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

In a previous communication,[1] it was shown that tuberculous guinea-pigs, rabbits and dogs react to intraperitoneal reinoculation with tubercle bacilli by causing rapid degenerative changes in the injected bacteria and a rapid decrease in their number, not observed in normal animals. The question now arose as to the mechanism of this heightened peritoneal resistance. From the similarity between this phenomenon and the Pfeiffer reaction

[1] These TRANSACTIONS, 1912, X, p. 30.

attempts were made to determine whether or not the specific antibodies, upon which the intraperitoneal lysis may be supposed to depend, are present in the circulating fluids.

To test this, guinea-pigs, rabbits and dogs were made tuberculous by inoculating them subcutaneously with tubercle bacilli. After an interval of from five to eight weeks, these animals were bled and their blood tested *in vitro* and *in vivo*. In a number of these experiments direct transfusion of the blood was made from the tuberculous animals into normal animals, an amount o blood often as great as three quarters of the total blood-volume being thus passed into the circulating system of the normal animals, the normal animals having been previously bled to free them as much as possible from normal blood. The transfused animals were subsequently tested by intraperitoneal injections of tubercle bacilli.

Neither in the test-tube experiments, nor in normal animals injected subcutaneously, intravenously or intraperitoneally with tuberculous serum, nor even in the normal animals directly transfused with large quantities of the unaltered blood of tuberculous animals, has the reaction thus far been obtained. The substances responsible for the heightened peritoneal resistance, therefore, apparently do not exist in appreciable quantities as circulating antibodies, at least at the stage of the disease studied. The heightened tuberculous resistance, therefore, is apparently due to substances held in fixed tissue cells.

Evidences of tuberculolytic substances have, however, been obtained in the peritoneal fluids of tuberculous guinea-pigs, soon after the introduction of tubercle bacilli. If these fluids are withdrawn, centrifuged free from formed elements and then introduced into the peritoneal cavities of normal guinea-pigs, they confer upon the normal peritoneal cavities a slight power of destroying tubercle bacilli. It is suggested, therefore, that fixed tuberculolysins are set free by the peritoneal cells in response to the presence of tubercle bacilli, and that these lysins account for the heightened resistance to intraperitoneal reinoculation with tubercle bacilli.

48 (744)

A preliminary communication on certain specific reactions exhibited by hay fever cases.

By GEORGE H. A. CLOWES.

[*From the Laboratory of Biological Chemistry, State Institute for the Study of Malignant Disease, Buffalo, New York.*]

Dunbar was the first to demonstrate conclusively that the European or spring variety of hay fever is caused by the pollen of timothy and other members of the graminaceæ family. He found, that, even in midwinter an aqueous extract of timothy pollen produced flushing and intense irritation when introduced into the eye of a susceptible individual, whilst normal individuals were entirely unaffected by such a procedure.

Sufferers from the American or autumnal form of hay fever (caused by the pollen of ragweed, golden rod, and other members of the Compositæ family) exhibit a similar sensitiveness when tested with aqueous extracts of ragweed pollen. This reaction is also strictly specific, and only those who suffer from both spring and autumnal hay fever react to the extracts obtained from both varieties of pollen. The amount of pollen extract required to produce flushing in the eyes of sensitive individuals varies from 1/20 of a c.c. of a solution of one in five hundred thousand to 1/20 of a c.c. of a solution of one in five hundred. This test can be utilized quantitatively and affords a fair index of the measure of immunity or resistance possessed by sensitized individuals.

A specific cutaneous reaction capable of quantitative application may be obtained by making a slight abrasion of the skin and applying a drop of pollen extract of suitable concentration. A large white welt similar to that resulting from a mosquito or bee bite accompanied by a distinct itching sensation develops on susceptible individuals within 15 minutes, while normal controls are unaffected. A small dose of pollen extract (1 c.c. of a one in a million for example) injected subcutaneously produces in highly sensitized individuals an itching or prickly sensation followed immediately by considerable swelling at the point of injection and redness extending sometimes over an area of several inches. The

injection of a somewhat larger dose (1 c.c. of a one in fifty thousand) caused a considerable swelling and pain, and small blisters resembling hives developed at the point of injection. This was followed by dizziness and a sense of general discomfort and a slight attack of hay fever was precipitated.

Specific precipitin and complement deviation reactions using pollen extracts as antigens were exhibited by certain cases before the commencement of the hay fever season. These reactions disappeared under treatment (see following paper) reappearing a few weeks later. Since the reactions in question, while quite definite, were not exhibited by all the cases, it is proposed to make them the subject of further investigation. The leukocyte count and body temperature were frequently above the normal in the course of an acute attack of hay fever. All attempts to induce hay fever symptoms in normal individuals by introducing into their eyes pollen extracts mixed with the serum or nasal secretions of sensitized individuals have failed. It may be concluded from these results that specific enzymes capable of splitting the pollen protein and liberating a toxic factor, or immune bodies capable of combining with the specific body present in the pollen extract to form a toxic combination are either not present or not demonstrable by this method in the blood or secretions of hay fever cases. Preliminary experiments which require confirmation would indicate rather that the serum of highly immunized hay fever cases when mixed with pollen extract may exert a slightly neutralizing effect on the latter, protecting the eyes of sensitive individuals to a certain extent from pollen toxin.

49 (745)

A preliminary communication on the treatment of autumnal hay fever by vaccination with an aqueous extract of the pollen of ragweed.

By GEORGE H. A. CLOWES.

[*From the Laboratory of Biological Chemistry, State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

Dunbar was the first to attempt active immunization against hay fever by vaccination with aqueous pollen extract. He used

too large a dose (about ten thousand times that which we have found most satisfactory), and the patient on whom he tried the experiment experienced such a violent anaphylactic shock that he decided to abandon direct vaccination, and subsequently resorted to passive procedures. Noon and Freeman succeeded in considerably alleviating the condition of sufferers from European or spring hay fever by injecting small doses of timothy pollen extract.

So far as we are aware no previous attempts have been made to immunize against American autumnal hay fever by vaccination. The extracts which we have employed for this purpose were prepared from the pollen of ragweed by one of the two following procedures: (1) That previously employed by Dunbar consisting of repeated freezing and thawing in a 5 per cent. aqueous suspension, and (2) an original method, which consists of precipitating the pollen with acetone and extracting with water.

A series of eight cases were vaccinated last summer, using doses ranging for the most part from 1 c.c. of a one in five million solution to 1 c.c. of a one in five hundred thousand. The size of the initial dose, the increase in the amount at each injection, the number of doses administered (ranging from three to twelve), and the time intervals (ranging from two to six days) were regulated by making frequent ophthalmic, cutaneous and blood tests and noting the measure of immunity indicated by these tests and the general condition of the patient. All the cases treated experienced a marked alleviation of general symptoms corresponding very closely with the physical tests. In the following table are recorded the initial resistance, maximum resistance, and resistance five months after completion of treatment exhibited by a series of cases. The figures represent the number of units of pollen toxin required to produce a definite ophthalmic reaction, one unit being the soluble constituents of one twenty-millionth of a gram of pollen. The figures included

RESISTANCE CLASSIFIED UNDER TREATMENT.

	Initial.	Maximum.	Five Months After Treatment.
Dr. C.	2	2,000 (40,000)	10
W.	40	2,000 (20,000)	20
Dr. Ch.	20	1,000	10
Dr. P.	40	2,000	10
Mrs. W.	2	1,000	

in brackets indicate the number of units required to produce an itching sensation in the eye which apparently runs considerably higher than the flushing point in highly immunized individuals. Extremely small doses (less than those recommended by Noon and Freeman in treating European hay fever) appear to give the best results. One case, for example, received four injections at intervals of four days, the amounts being .12, .18, .24, and .36 c.c. of a one in one hundred thousand extract of ragweed pollen, and showed at the end of this period a fifty-fold increase in resistance. Another case recently immunized against spring hay fever for experimental purposes, received three doses of timothy pollen extract, 1 c.c., 1½ c.c. and 2 c.c. of a solution of one in five million at intervals of six days, and at the end of this period exhibited a hundred-fold increase in resistance. In another case in which the resistance had already been raised by previous treatment a thousand-fold, a dose of 2½ c.c. of a one in one hundred thousand solution produced urticaria, dizziness and other unpleasant symptoms referred to in the preceding paper and obviously constituted an overdose. The employment of doses in excess of 1 c.c. of one in one hundred thousand is unnecessary, possibly dangerous and certainly to be avoided. That the changes noted above are not due solely to the development of natural immunity in the course of the disease is proved by the fact that three cases after experiencing typical hay fever symptoms for two or three weeks were immunized in the middle of the season and immediately showed a marked improvement, and three cases treated long after the close of the season rapidly developed a greatly increased resistance to the pollen extract employed. The specific nature of the immunity developed by injecting specific pollen extracts was proved in two cases suffering from both spring and autumnal hay fever, one of whom, Mrs. K., was recently partially immunized against timothy without changing the ragweed sensitiveness, the other, Miss R., being similarly immunized against ragweed without affecting the timothy resistance (see table).

		Initial.	Maximum.			Initial.	Maximum.
Mrs. K.....	R.	200	200	Miss R.....	R	200	800
	T	200	400		T	200	200

50 (746)

Abderhalden's biological test of pregnancy.

By P. F. WILLIAMS and R. M. PEARCE.

[*From the John Herr Musser Department of Research Medicine, University of Pennsylvania.*]

SUMMARY.

The use of Abderhalden's test for pregnancy, employing the dialysis method and the ninhydrin color reaction has given positive results with each of 28 sera from pregnant women and with eight from women in the post-partum period, including one abortion. The test has never been negative in a known pregnancy. On the other hand, the serum of pregnancy reacts with tissues (kidney, heart, uterus) other than placenta. Also sera of two cases of nephritis, one of tabes and one of infection (carbuncle), and occasionally of some individuals in apparent perfect health have given the reaction with placenta and other tissues.

In the use of Abderhalden's dialysis method we have found the ninhydrin reaction far superior to the biuret reaction. It is also important that Schleicher and Schull's smaller dialysis sacks should be used rather than the fish skin membranes originally recommended.

Results as satisfactory as those obtained by dialysis are obtained by mixing tissue and serum in tubes and after incubating for 24 hours testing the filtrate obtained on coagulation by heat and acetic acid with ninhydrin.

Inactivation of the serum causes a great diminution in the degree of reaction, but does not cause it to disappear entirely. At zero temperature no reaction occurs. The power of a serum to cause the reaction persists when the serum is kept under proper conditions of temperature for at least 7 days.

As the result of our studies we feel that this test cannot be accepted as an accurate clinical method until it has been more thoroughly investigated and the possible sources of error corrected. This conclusion however applies only to Abderhalden's dialysis method and not to his optical method with which we have had no experience.

51 (747)

The Kernplasmarelation during the life of a pedigreed race of Oxytricha fallax.

By LORANDE LOSS WOODRUFF.

[*Sheffield Biological Laboratory, Yale University.*]

The following conclusions were reached from a study of cells during periods of characteristically different reproductive activity of an 860 generation pedigreed race of *Oxytricha fallax:*

1. A wide variation in the size of the cells and of the nuclei occurs at all periods of the life of the race.

2. The mean size of the cell is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.

3. The mean size of the nucleus is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.

4. The Kernplasmarelation of individual cells shows a wide variation at all periods of the life of the race.

5. The mean proportion of nuclear to cytoplasmic material is highest during the period of greatest reproductive activity.

6. The size of the cell and the size of the nucleus as well as the Kernplasmarelation are interpreted as an incidental result rather than as a cause of the rate of cell division.

52 (748)

The reaction between oxygen and hemoglobin.

By E. E. BUTTERFIELD.

[*From the Rockefeller Institute for Medical Research.*]

There are at present four different views as to the nature of the absorption of oxygen by blood.

The first and oldest view is expressed by the reation,

1 mol. hemoglobin + 1 mol. oxygen $\rightleftharpoons$ 1 mol. oxyhemoglobin.

According to the law of mass action one would have

$$ab = kc, \qquad (1)$$

in which a represents the concentration of hemoglobin in solution, b the oxygen concentration, c the concentration of oxyhemoglobin, and k a constant.

The second view is that of Bohr. Bohr assumed that hemoglobin in aqueous solution is hydrolytically split into globin + Fe-component, and that the Fe-component combines reversibly with oxygen. Bohr's formulation of this hypothesis leads to an equation of the fourth degree. Without going into details it will suffice to call attention to the main points of Bohr's work as far as we are concerned here. They are, in addition to the assumed hydrolysis of hemoglobin in aqueous solution, first, the inapplicability of formula (1) to his results and secondly, the variation of the ratio $\frac{\text{oxygen absorbed}}{\text{total hemoglobin concentration}}$ with the total hemoglobin concentration[1] at constant pressure.

The third view is that of Wo. Ostwald who applied the older adsorption formula,

$$x = kc^n, \qquad (2)$$

in which x = oxygen adsorbed, c = oxygen concentration, k and n are constants, to some of the data of Paul Bert and Loewy and found that log x and log c plotted in rectangular coördinates gave a straight line.

The fourth and last view is that brought forward by Manchot, who found that the quantity of oxygen or CO absorbed by 100 c.c. blood, laked or unlaked, varied with the dilution, *i. e.*, with the total hemoglobin concentration. On 10-fold dilution this quantity approached a maximum value of 2 mol. oxygen to 1 mol. hemoglobin.

The experiments which I have to report were carried out during the last 4 years in the course of other studies. These experiments are restricted to the influence of change in the total hemoglobin concentration on the amount of gas bound pro gram hemoblogin at constant gas pressure. The results also hold only for hemoglobin in solution, *i. e.*, aqueous solutions of crystalline oxyhemoglobin or centrifuged red corpuscles dissolved in H_2O. It is to be emphasized that the few experiments cited here have been

[1] By total hemoglobin concentration is meant here and in the following the total weight of blood coloring matter (hemoglobin + oxyhemoglobin) in unit volume.

selected solely with reference to the lowest and highest total hemoglobin concentration in each series. Four entirely different methods were employed and they all lead to the same result.

TABLE I.

Absorption of CO. Hemoglobin spectrophotometrically.

	λ	c	$\frac{CO}{Hb}$	
I.	654	3.59	1.29	
	658	4.86	1.30	Laked ox blood.
	668	2.88	1.29	
	616	5.40	1.27	Laked human blood.

Reduction with palladium-hydrogen. Hemoglobin gravimetrically.

	λ	c	$\frac{O_2}{Hb}$	
II.	156	1.23	1.09	
	154	11.50	1.08	Oxyhemoglobin from ox blood.

Oxygen capacity by ferricyanide method. Hemoglobin spectrophotometrically.

	c	$\frac{O_2}{Hb}$	
III.	1.64	1.37	
	4.27	1.40	Laked rabbit blood.

Spectrophotometric.

	Dilution	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$	
	2	1.12	
	10	1.13	Laked ox blood.
	1.5	1.13	Laked ox blood after extraction with ether.
	10	1.13	
IV.	200	1.13	

	c	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$	
	1.14	1.13	
	7.00	1.13	Oxyhemoglobin from ox blood.

The first group of experiments was carried out with laked ox blood and with laked human blood. The volume of CO absorbed by oxygen-free blood ($(NH_2)_2.H_2O$ and vacuum pump) was determined. The quantity of CO physically absorbed was deducted from the total volume, using the coefficient of absorption for CO in H_2O at the temperature of the experiment (20° C.) for the calculation. Total hemoglobin concentration determined spectrophotometrically. It will be seen from the table that for a small

range of concentration (2-fold) the CO absorption pro gram hemoglobin is practically constant for laked ox blood or human blood. (In the table, c = hemoglobin concentration, gram in 100 c.c., CO = volume CO absorbed by hemoglobin, Hb. = total hemoglobin quantity in grams, and p = pressure of CO in mm.) The deviations are no greater than could be accounted for by the slight decrease of the solubility of CO in hemoglobin solutions of ascending concentrations. It will be noticed that at a CO pressure of 650 mm. the limit 1 mol. CO to 1 mol. hemoglobin is almost reached (theor. 1.34 c.c. CO pro gram hemoglobin). The difference again is in all probability due to the difference in coefficients of absorption of CO in H_2O and in blood of the concentrations studied. The reason for the good agreement between the values for ox blood and human blood rests in the identity of the optical constants for human hemoglobin and ox hemoglobin.

The second series of results was obtained by the method of Paal. Reduction of dialyzed solution of oxyhemoglobin, saturated with air at the prevailing atmospheric pressure, in an atmosphere of hydrogen in the presence of colloidal palladium. The quantity of hydrogen absorbed was measured. It was assumed that under the conditions of the experiment oxyhemoglobin + H_2 = H_2O + hemoglobin. Previously the quantity of H_2 absorbed by 0.1 gram Pd in 110 c.c. H_2O was determined. This was deducted from the total H_2 absorbed in the experiments with hemoglobin. In all of these experiments 0.1 gram Pd and 110 c.c. solution were constantly used. Hemoglobin concentration determined gravimetrically. It will be seen from the table that for an almost 10-fold variation in the hemoglobin concentration the quantity of O_2 pro gram Hb. remains constant. The value is considerably lower than for CO at high pressures. It is not necessary to go into the various possibilities (incomplete reaction, adsorption of hemoglobin by Pd, etc.) which may account for this difference. It suffices here to call attention to the fact that the quantity of O_2 given off pro gram Hb. to a reducing agent is independent of the total hemoglobin concentration if the solutions have been previously saturated at the same partial pressure of oxygen.

The third series[1] was obtained by the ferricyanide method of

[1] From an unpublished study on the action of pneumococcus on blood, by Dr. F. W. Peabody and myself.

Haldane and Barcroft with laked rabbit saturated with oxygen at the prevailing partial pressure in the air. Total hemoglobin concentration determined spectrophotometrically, using the constants of oxyhemoglobin from ox blood. Here again for a 3-fold variation in concentration O_2/Hb. is constant. The apparent slight excess over 1 mol. O_2 to 1 mol. Hb. is probably due to a slight difference in the optical constants of oxyhemoglobin from rabbit blood and oxyhemoglobin from ox blood.

The fourth and last series was obtained from measurements of the light absorption of laked ox blood and oxyhemoglobin from ox blood at the wave-lengths 577, 579 $\mu\mu$ (double line, mercury arc) and 546 $\mu\mu$. Log (I_1/I_1') is the log of the ratio of initial and final intensities in the solution at 577, 579 $\mu\mu$, log (I_2/I_2') the log of the corresponding ratio at 546 $\mu\mu$. Between the quotient of the logs of these ratios and the concentration of hemoglobin (oxygen-free) in the presence of oxyhemoglobin there exists the following relation:

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{(\beta_1 - \alpha_1)x + \alpha_1}{(\beta_2 - \alpha_2)x + \alpha_2}, \qquad (3)$$

in which x = relative concentration of hemoglobin, α_1, α_2, β_1, β_2 constants which can be determined experimentally. If $\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ remains constant on dilution then x must be constant. That is if $\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ remains constant on dilution at constant O_2 pressure the relative composition of the solution (oxyhemoglobin and hemoglobin) also remains constant.

We will now apply these results to the existing views on the nature of the absorption of oxygen by hemoglobin. Several considerations not embodied in the table will also be of aid in selecting the most probable formula.

The Bohr formula falls out for the following reasons:

1. When the globin is split off from the hemoglobin molecule the iron-component can be identified as hematin (or its reduction product, hemochromogen), which has a characteristic spectrum. All of our spectroscopic and spectrophotometric evidence is against the presence of hematin or hemochromogen in normal blood or in solutions of freshly prepared oxyhemoglobin. I have recently been able to split hemoglobin in glycerin solution by means of $(NH_2)_2.H_2O$ reversibly into globin + hemochromogen. The reaction, however, only takes place under special conditions and is always accompanied by sharp spectral changes.

2. Granted for the moment that hemoglobin is hydrolyzed in aqueous solution. Then it must be possible to shift the equilibrium by changing the concentration of H· or OH′ ions. Following experiments[1] show that such a shift of equilibrium does not occur or at least if it does it has no influence on the combination of oxygen with hemoglobin at constant pressure. Similar results were obtained with KOH. On increasing the H· or OH′ concentration beyond the quantities given in the table a constitutive change occurs in the oxyhemoglobin molecule; methemoglobin is formed.

TABLE II.

	c	O_2-Capacity.	$\frac{O_2}{Hb}$	Quantity of $n/10$ HCl Added to 4.5 c.c. Washed Rabbit Corpuscles in c.c.
I	4.27	5.97	1.40	0.0
II	4.47	6.25	1.40	0.5
III	4.48	6.37	1.42	1.0
IV	4.40	6.35	1.44	2.0

3. From Bohr's experiments and formula the quantity of O_2 absorbed pro gram hemoglobin is a function of the total hemoglobin concentration. According to the experiments here presented the quantity of O_2 bound pro gram hemoglobin is independent of the total hemoglobin concentration at constant gas pressure.

The arguments 2 and 3 against the Bohr formula also hold for Manchot's work. In addition it is to be noted that in our experiments the limit value for the oxygen (or CO) absorption is 1 mol. oxygen to 1 mol. hemoglobin. In no case does it approach 2 mol.

[1] From the pneumococcus study by Dr. Peabody and myself.

The spectrophotometric data are conclusively against Manchot's views. Manchot reached the conclusion that undiluted blood contains about 33 per cent. hemoglobin uncombined with oxygen, while at 10-fold dilution the amount of uncombined hemoglobin approaches zero. From the spectrophotometric data it follows that x (or $100x$, the percentage of uncombined hemoglobin) is practically independent of dilution at constant oxygen pressure.

Of the four views mentioned in the beginning, this leaves the choice between the mass action formula for a monomolecular chemical reaction and the adsorption formula. Our experimental results furnish little aid in this choice, except the fact that the limit value of oxygen (or CO) absorption is 1 mol. gas for each mol. hemoglobin. This would indicate, of course, a monomolecular reaction. A very strong argument against the adsorption theory is the fact that combination of hemoglobin with oxygen is accompanied by a sharp change in the spectrum, which as far as I know can only be interpreted as constitutive molecular change. Not having any data of my own suitable for a test of the adsorption formula, I have taken the data of Bohr, Hüfner, and Loewy. In no case is $m \log c + \log k$ a straight line function of $\log x$. Even from Loewy's numerical data it is impossible to find support for the adsorption theory—it will be recalled that Wo. Ostwald bases his claim for the adsorption of oxygen by blood chiefly on a curve of Loewy's. The formula $x = kc^n$ is only of limited application to adsorption phenomena. On trying the more general formula of Arrhenius on the same data one finds that k is not constant. So on the whole it may be said that there is no evidence to support the view that oxygen is *adsorbed* by hemoglobin. It is possible that adsorption may play a rôle in the taking up of oxygen by intact red corpuscles, but there too the chief phenomenon is more probably the chemical combination of oxygen with hemoglobin.

This leaves finally the first and oldest view of reaction between oxygen and hemoglobin as the only one of the four which is at the same time tenable and compatible with the results here presented. These results are entirely in accord with formula (1), b being practically constant in each series (or varying slightly with the solubility of the gas in solutions of different total hemoglobin concentration). We would have then,

$$\frac{\text{conc. hemoglobin}}{\text{conc. oxyhemoglobin}} = \text{const.},$$

which is a uniform result at constant pressure and temperature, obtained by four different methods. It is necessary in closing to call attention to the fact that in all experiments the gas pressures were relatively high and that under these conditions one is working in the neighborhood of maximum saturation of hemoglobin with CO or oxygen.

53 (749)

The effects of stimulation of the labyrinth of the ear in the living animal. (With demonstration.)

By **J. GORDON WILSON** and **F. H. PIKE.**

It may be shown that if one sits in a moving railway train and observes objects out of the window, they may seem to be moving in a direction opposite to that of the train. If one notices the movements of one's eyes in observing these objects, it will be seen that the eyes move slowly backward, opposite to the direction in which the train is going, and more quickly return to the median position, the quick movement of the eye being in the same direction as the movement of the train. We may express this relation between eye-movements and apparent movement of external objects or real passive movement of the one's own body by the statement that the apparent movement of external objects is in the direction of the slow deviation of the eyes, and the real passive movement of one's body is in the direction of the quick movement of the eyes. The otic labyrinth is not involved in these reactions. We may extend this relationship still further and say that under whatever conditions of this kind, with one possible exception,[1] one may be placed the movement, either real or apparent, of external objects is in the direction of the slow deviation of the eyes, and passive movement, either real or apparent, of one's own body is in the direction of the quick movement of the eyes. While many of the facts have long been known, we have not seen any general expression of the relationships in terms of the slow and quick eye movements.

[1] Holt, E. B., *Psychological Review*, 1909, XVI, p. 388.

Stimulation of the otic labyrinth in a number of different ways, including rotation about a particular axis of the head, evokes movements of the eyes, and sensations of apparent movement of external objects or of one's own body, or both. The movement of the eyes is slow to the stimulated side if a weak stimulus is used, and slow to the opposite side if a strong stimulus is applied.[1] Here also the real or apparent movement of external objects is in the direction of the slow deviation of the eyes, and the apparent passive movement of the body, or the real passive movement, if rotation occurs, is in the direction of the quick movement of the eyes, if it occurs. This quick movement of the eyes is lacking in some lower forms, such as the turtle.

When, by any means of labyrinthine stimulation, sensation of apparent (passive) movement of the body is produced in an animal at rest, the active compensatory movement of the animal is opposite in direction to that of the apparent movement of the body, and is in the direction of the slow movement of the eyes. This is the basis of the explanation of the fact, observed clinically, that patients with labyrinthine lesions fall or make other body movements in the direction of the slow deviation of the eyes, a relationship not previously sufficiently emphasized.

In electrical stimulation of the labyrinth by the direct current, the animal (turtle) moves toward the side on which the anode is placed. Reversing the direction of the current changes the direction of movement of the animal.

We may give a description of the passive movements, either real or apparent, and the active or compensatory movements of the body, under these various conditions, in terms of eye movements by the statement that the passive movements are in the direction of the quick movement of the eyes, and the active compensatory movements are in the direction of the slow deviation of the eyes.

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[1] Wilson and Pike, *Phil. Trans. Royal Society*, London, Series *B*, 1912, Vol. 203, p. 135.

54 (750)

The pancreatic lipase of infants in acute intestinal disturbances.

By ALFRED F. HESS.

[*Research Laboratory, Dept. of Health, N. Y. C.*]

In the former communication which considered the pancreatic secretions in chronic malnutrition in infants, it was found that various ferments of the gland are normally secreted even in advanced instances of marasmus or atrophy. In the present study of an *acute* disease, of acute intestinal indigestion or alimentary intoxication, which was carried out also by the direct method, by the use of the duodenal catheter the lipase was found deficient, although the two other pancreatic ferments were present in considerable amount. The deficiency of lipase seemed to some degree characteristic of this disturbance; it is not a general characteristic of all febrile conditions and was not met with in pneumonia or empyema. It is possible that the lack of lipolytic activity in this disease should be correlated with the clinical manifestation of fat intolerance, and the metabolic studies showing a deficient absorption of fat.

55 (751)

The influence of protein concentration upon the absorption of antibodies from the subcutaneous tissues.

By W. H. PARK, L. W. FAMULENER and E. J. BANZHAF.

[*From the Laboratories of the Department of Health, New York City.*]

The experiments so far completed indicate that the concentration of protein up to double its normal amount in an antitoxic or agglutinating serum or globulin solution has but little influence upon the absorption of the contained antitoxin or agglutinin from the subcutaneous tissues of man or animals unless the increased concentration of protein together with other substances gives rise to a greater local inflammatory reaction. The absorption of agglutinin was markedly less in a number of rabbits in which the subcutaneous injections of the high proteid solutions were followed by infiltration and necrosis of the adjacent tissues.

The high proteid concentration did not appreciably lessen the amount or rapidity of absorption, when no such reaction in the tissues took place. Contrary to the conclusion of Walbum the results obtained in four healthy men did not show any appreciable difference in absorption of antitoxin from an antitoxic globulin solution, the proteid concentration of which was equal to that of normal horse serum and one in which the concentration was double that amount.

56 (752)

The influence of the vagus nerves on the faradized auricles in the dog's heart.

By G. CANBY ROBINSON.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

The effect of vagus stimulation on the abnormal cardiac activity set up by faradization of the dog's auricle was studied in twenty-three experiments. Faradization of the auricles threw them into a tumultuous activity which in fifteen of the experiments persisted after faradization was discontinued from five minutes to over an hour. In these experiments opportunities were afforded for studying the nature of the abnormal auricular activity set up by faradization and for determining what effect stimulation of each vagus nerve had upon it. In several experiments the effect of cutting the vagi while the abnormal activity was present was observed. In eight experiments in which the abnormal activity could not be established independently, the effect of vagus stimulation was observed by beginning it before ending the faradization of the auricles. When this was done the abnormal auricular activity usually continued until after the end of vagus stimulation and was affected in the same manner as the continuous or established tumultuous activity.

The auricular activity resulting from auricular faradization consisted in very rapid movements, apparently contractions of the whole auricles, which were sufficient to produce definite movements of the recording tambour attached to the auricular myocardiograph. Beside this rapid auricular tachycardia, fine fibril-

latory movements in the various fibers could be seen. When the right vagus was stimulated with a faradic current of moderate strength, the coarser movements ceased and the typical fine fibrillations persisted, and when the stimulation was removed the coarser movements could be seen definitely, gradually returning and being coexistent with the fine fibrillation. This effect produced a change in the electrocardiogram and the undulations representing auricular activity became more rapid, blurred and often almost disappeared. This characteristic change in the electrocardiograms occurred in 89.5 per cent. of the experiments.

When the left vagus was stimulated the coarser movements of the auricles were not disturbed, but appeared sometimes perhaps even more distinctly than before. It was difficult to determine with certainty whether the fibrillatory movements ceased or were influenced. There was a definite difference in the electrocardiograms when the right and left vagi were stimulated during the abnormal auricular activity in 70.5 per cent. of the experiments, the larger waves of auricular activity being much less or not at all disturbed by stimulation of the left nerve.

Thus the difference between the action of the two vagi in the dog, pointed out by Cohn,[1] is further extended.

Cutting the vagi after the establishment of the abnormal auricular activity had little or no effect upon it, but the ventricular rate was sometimes much increased. Vagus stimulation increased the susceptibility of the auricles to faradization, and in four experiments the abnormal auricular activity could be made to continue after the faradization was stopped only by stimulating the vagi synchronously with or for a few seconds after faradization. In two experiments an auricular activity identical with that following faradization was set up by right vagus stimulation alone.

The normal sequential beat is also often restored by vagus stimulation. It replaces the abnormal auricular activity not during, but a few seconds after the termination of vagus stimulation. Left vagus stimulation seems somewhat more effectual in producing this effect than right vagus stimulation.

[1] Cohn, *Jour. Exper. Med.*, 1912, XVI, 732.

57 (753)

Prolonged complete heart-block, without lesion of the bundle of His and with frequent changes in the idio-ventricular electrical complexes.

By **B. S. OPPENHEIMER** and **H. B. WILLIAMS.**

[*From the Department of Physiology, Columbia University, New York.*]

Electrocardiograms were obtained at intervals from an old hemiplegic patient who was known to have complete heart-block from February 26, 1912, up to the day of his death, December 31 of the same year. During many months of this period he had pronounced Cheyne-Stokes respiration. The interest in this case lies in the fact that there were frequent changes in the electrical complexes of the ventricular beat and correlated with this the fact that histological examination revealed no organic lesion to account for the block, in the auriculo-nodal junction, the node of Tawara, or the main stem and its branches. The nodal artery was sclerotic.

The variations in the ventricular complexes were seen not only from one examination to the next, but often from beat to beat.

The waves *Q*, *R*, *S* and *T* all showed variations; for example with leads I and II the wave *R* was sometimes upright, sometimes inverted. With lead III the wave *R* was always inverted.

The auricular rate was strikingly reduced during the dyspneic period (44.49 beats per minute), as compared with its rate during apnea (94.17 beats per minute). The ventricular rate was only slightly reduced during dyspnea (30.46 beats per minute as compared with 31.25 beats per minute). In other words the vagus still had a marked chronotropic effect on the auricle and little if any on the ventricle. Two atropin tests were made during which the heartblock was not relieved; and, again, the auricular rate was decidedly increased, the ventricular rate very slightly so.

To explain the divergent types of ventricular complexes we may consider the possibility that the intrinsic ventricular pacemaker was frequently shifting, or that the different impulses

started at the same point and traveled either along different routes, or at varying rates along the same route.

Complete heart-block without anatomical lesion in the auriculo-ventricular system may possibly be of neurogenic or of circulatory origin, or it may be ascribed to chemical agents, to asphyxia, or to some hindrance to the passage of impulses from the terminal arborizations of the conducting system to the ventricular musculature.

A previous example of possible functional heart-block was reported by Dr. Alfred Cohn.[1] In his case of transient complete dissociation showing constantly varying ventricular complexes, the patient recovered, so that there was no opportunity of determining whether or not there was an organic lesion in the auriculo-ventricular system.

58 (754)

Methods for the production of temporary valvular lesions.

By CARL J. WIGGERS and EUGENE F. DU BOIS.

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

Experimental valvular lesions have been induced by a number of investigators—stenoses by tightening of ligatures or clamps about the valvular orifices, insufficiencies by tearing of valves with sounds and glass rods or by cutting with specially constructed valvulotomes. Such experimental stenoses may, if desired, be temporary, and normal circulatory conditions may be subsequently reëstablished. Experimental insufficiencies such as have been described, must, owing to the traumatic nature of the lesion, be permanent. As no method for the production of temporary insufficiencies has apparently been described, the following method, which also permits a study of the intraventricular pressure changes, was devised.

Method.—A curved metal catheter (22 cm. long, internal diameter 6 mm.) having toward the tip one or two openings (6 mm. in diameter) and three centimeters from the tip a longi-

[1] Cohn, PROC. SOC. FOR EXPER. BIOL. AND MED., Vol. IX, No. 2, p. 24, December, 1911.

tudinal slot (6 mm. wide and 25 mm. long) is fitted with a lubricated rubber tube (4 mm. internal diameter) so as to occlude the longitudinal slot. To produce aortic insufficiency, the metal catheter with its rubber obturator is introduced, free from air and without hemorrhage, into the left subclavian artery and aorta. The catheter is so adjusted by palpation that the valves close about it near the middle of the occluded slot in the catheter. The inner rubber tube may now be connected directly with a manometer.[1] Aortic pressure records with Frank's manometer show that a catheter of such size that it can be introduced into the subclavian does not impede the systolic discharge so as to cause stenosis in the physiological sense. By drawing out the obturating rubber tube to such an extent that the slot is opened, a valveless circuit with a minimal resistance is established between aorta and ventricle. The intraventricular pressure may still be recorded.

Upon entirely withdrawing the metal catheter, normal conditions are restored. By inserting the catheter through the auricular appendage, the method may be used to induce mitral regurgitation. Postmortem examination of six hearts showed no damage of the valves.

The advantages of being able to produce temporary valvular insufficiencies are several:

1. It permits the consecutive demonstration of all valvular lesions and their circulatory effects on the same animal. In such a series of demonstrations we produce mitral and tricuspid stenosis by simple invagination of the auricular wall into the auricular ventricular openings with a finger.

2. By subsequently reëstablishing normal conditions, it permits a control of the reflex circulatory and respiratory disturbance incidental to the production of experimental valvular lesions. It is quite possible for such reflex effects to entirely overshadow or complicate the mechanical effect of the lesion and, unless so controlled, the results cannot be considered comparable to clinical lesions.

[1] In the case of Frank's optical manometer, the connection can be made rigid and without elastic connection.

59 (755)

The influence of the central nervous system in regeneration of an annelid worm.

By A. J. GOLDFARB.

[*From the Biologic Laboratories of the College of the City of New York.*]

In a previous investigation, it was found that the Morgan and Nussbaum methods of operating worms resulted in a large mortality, excessive injury, retarded healing and little regeneration. The same results were obtained with the marine annelid worm *Amphinoma pacifica.*

By the method used successfully with *Lumbricus* 90 *Amphinomas* were operated in such a manner that the nerve cord was completely removed from 2 to 6 or more segments next the amputated level and with little or no injury to adjoining tissues. Over 151 worms served as controls.

Regeneration was limited to definite levels. Within these regions the head or the tail was regenerated in most of the worms, even though the nerve cord had been removed. On subsequent examination of serial sections it was found that in one group of worms, the nerve cord had regenerated from the broken end as far as the amputated level. Some of these worms had begun to proliferate new tissues, others showed no sign of proliferation. In a second large group, the regenerated nerve cord of the new head was continuous with the old nerve cord.

In a third group, the old nerve cord had grown a few ganglia towards the amputated level, the remaining segments were entirely devoid of nerve cord. Nevertheless the head had been regenerated with its typical supraesophageal ganglia or "brain," its commissures and even ventral nerve ganglion which grew posteriorly towards the old cord. This group demonstrated that *Amphinoma* as well as *Lumbricus*, and other adult organisms could regenerate the missing organ without the contact of or stimulation from the central nervous system.

60 (756)

Changes in concentration of sea water and their influence upon regeneration.

By A. J. GOLDFARB.

[*From the Biologic Laboratories of the College of the City of New York.*]

The regeneration under changed densities of sea water was observed under conditions that ensured the elimination or uniformity of associated factors such as size of medusæ, volume, surface and depth of solutions, extent of injury, level of amputation, temperature, crowding, aeration, etc. Furthermore, the dilute solutions were made with water containing a known quantity of sea salts, and the concentrated solutions were made by slow evaporation in the sun's heat, which corrected certain errors in previous experiments of this nature. The results were checked by repeated observations, plotted for intervals of 14, 24 and 30 days, after amputation of the arms of *Cassiopea xamanacha.*

These medusæ lived in solutions ranging from 40 to 153 per centum of the salts of sea water. They regenerated however in 50 to 133 per cent. solutions. The regeneration of normal arms and supernumerary arms was limited further to 75 to 105 per cent. solutions. Beyond these limits regeneration was atypic. Optimum regeneration occurred not in sea water but in sea water diluted 95 to 90 per cent. With increasing dilution the amount regenerated was diminished very slowly, with increasing concentration very rapidly. The subsequent examinations revealed the fact that in the sub-optimum solutions the arms regenerated absolutely and relatively faster than in any of the other solutions, correspondingly changing the character of the curve.

When the results were compared with those of Loeb it was found that both the hydroid *Endendrium* of Woods Hole, Mass., as well as *Cassiopea* of Dry Tortugas, Fla., differed radically from the classic experiments of Loeb on *Tubularia* of Naples, in respect to the range of solutions in which animals lived or regenerated, the optimum solutions, the normality of the regenerated parts and the character of the curve. It is altogether probable that Loeb's curve is limited to *Tubularia* of Naples and does not

represent the behavior of organisms to changes of density of sea water, and that the differences in the behavior of these three organisms can hardly be correlated with the differences in concentration of the sea water in which they normally live.

61 (757)

Variations in the amount of transformed atoxyl (trypanotoxyl) produced by varying the strength of atoxyl incubated with blood.

By **B. T. TERRY.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

A 10 per cent. solution of atoxyl in blood incubated for 1 to 3 hours at 37° C. is more than 10 times as toxic for *Trypanosoma brucei* as a 1 per cent. solution in blood similarly incubated. To avoid misleading results due to the transforming action of red blood corpuscles upon unaltered atoxyl continuing after the dilutions are made, immediately after incubation all red blood corpuscles should be removed by centrifugalization from the fluid containing the transformed atoxyl.

62 (758)

Some observations on bacteria of the duodenum.

By **W. J. MACNEAL** and **A. F. CHACE.**

[*From the Laboratories of the New York Post-Graduate Medical School and Hospital.*]

The duodenal tube was sterilized by boiling in water for ten minutes and the lower end was covered with a tightly-fitting gelatin capsule which had been soaked in alcohol for several days. The gelatin capsule was finally coated with shellac and dried. The tube was ordinarily given late at night and the sample of fluid aspirated on the following morning, usually without any food being taken in the interval. In a few cases the fluid was obtained an hour after giving an Ewald test meal.

The study of the fluid included inspection, direct microscopic count of the bacterial cells, plating of measured amounts on litmus lactose agar and ascitic-fluid agar, separation cultures of measured quantities in tall tubes of ascitic-fluid agar and fermentation-tube cultures of measured quantities in dextrose broth and lactose broth. In addition, a portion of the fluid was heated to 80° C. for ten minutes and inoculated in measured quantities into tall tubes of glucose ascitic-fluid agar and into fermentation tubes of glucose broth and lactose broth.

Thirty-five samples were studied, of which the first nine were unsatisfactory because of defects in technic. The results obtained on the remaining twenty-six serve as a basis for this report.

The results can be presented here only in summary form. In general, the number of bacterial cells seen microscopically varied from 600,000 to 960,000,000 per c.c. and their number bore no evident relation to the number of colonies obtained in cultures nor to the clinical condition of the patient. On the other hand, the results of the culture work indicate that the normal duodenal fluid is practically free from living bacteria when food is absent, and that the number of cultivable bacteria obtained in a given case is a rough index of the digestive derangement. Gas-forming microbes developed in the fermentation tubes in ten of the twenty-six fluids examined, all from cases with gastro-intestinal disturbance. Bacterial spores capable of resisting a temperature of 80° C. for twenty minutes were not found in any examination.

One hundred ten subcultures were isolated and studied. Forty-three of these were Gram-positive, non-liquefying cocci. These were isolated from a majority of the samples and doubtless are the organisms most frequently present in health and in slight disturbances. Gram-negative, gas-forming bacilli which liquefy gelatin were isolated from five of the twenty-six fluids, all derived from cases of severe gastro-intestinal disorder. In some cases these bacilli were very abundant. Liquefying cocci, yeasts and various kinds of bacilli were isolated in less abundance. Only three of the 110 strains belonged in the *B. coli* group. Four of the twelve strains isolated from the one case of typhoid relapse proved to be *B. typhosus*.

63 (759)

The effect of morphine on the mechanism of the dog's heart after removal of one vagus nerve.

By ALFRED E. COHN.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

When injected intravenously, morphine was shown by v. Egmond to cause cardiac arhythmia in dogs. Einthoven and Meek and Eyster studied the results of such injections electrocardiographically. Einthoven concluded that these were due to stimulation of the vagus centers. He found complete lack of uniformity in the results. Meek and Eyster believed the effects to be due to disturbances in conduction between sinus and auricle and between auricles and ventricles.

The similarity of some of Einthoven's curves to those resulting from faradic stimulation of the right vagus nerve and of others from stimulation of the left nerve rendered it probable that morphine sometimes had a preponderating influence on the right and at others on the left vagus nerve and center. Experiments were accordingly carried out in twelve dogs, in six of which the right and in six of which the left vagus nerves were removed aseptically. In 3 morphine was injected before operation. Registration was galvanometric. In three dogs (one right during three attempts and two left during two and three attempts) arhythmia characteristic of morphine poisoning was not obtained.[1] In three it was obtained on the first attempt, in five on the second and in one on the third. In five right vagus and in four left vagus dogs injection succeeded and the resulting arhythmias were directly comparable to those obtained on faradic stimulation. In the right vagus dogs the auricles, except for occasional contractions which escaped at long intervals, and also the ventricles ceased to beat. The circulation was carried on by ectopic ventricular contractions. That there was no defect in conduction was shown in a number of ways. In the left vagus dogs the auricles were merely slowed. There was depression in A-V conduction in all of them,—in three

[1] The dogs are named according to the nerve retained.

it was of moderate degree and resulted in an As-Vs ratio of 2 : 1 or 3 : 1. In another it was severe; except occasionally, the ventricles received no impulses from auricular contractions, but maintained the circulation by idioventricular contractions in complete dissociation with those of the auricles. All the dogs recovered.

The conclusions are warranted: that morphine stimulation of the vagus center in dogs having one vagus nerve only has the same effect as faradic stimulation of the same nerve in other dogs; that the results of the morphine method substantiates a former conclusion based on faradic stimulation that characteristic differences between the two vagi exist; and that the lack of uniformity found by Einthoven in the arhythmia of the heart obtained on morphine injections is only apparent and is capable of analysis.

64 (760)

The influence of temperature on the minimal dose of strychnin in frogs and on the time of onset of tetanus.

By T. S. GITHENS.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Rana pipiens were used exclusively and all injections were given in the dorsal lymph sac. Temperatures from 40° F. to 80° F. were studied. With doses of 0.0006 mg. per gm. (about 0.02 mg. for a 30 gm. frog) tetanus was constantly obtained at all temperatures. With doses of 0.0005 mg. per gm. (about 0.015 mg. per frog) strong tetanus was obtained constantly at temperatures about 40° F. and about 80° F. It was occasionally seen at 55° F. and never at temperatures from 65° to 75° F. With doses of 0.0003 mg. per gm. strong tetanus was constant about 40° F. and occurred frequently at 80° F., but was never seen at temperatures from 55° F. to 70° F.

In regard to the time elapsing before tetanus; with minimal doses it is very variable but on the whole is less the higher the temperature. Thus with a dose of 0.0006 mg. per gm. tetanus came on at 40° after ½ to 4 hours; at 55° after 1 to 2¼ hours; at 70° after ¾ to 2 hours; and at 85° after ½ to 1 hour. With a

dose of 0.001 mg. per gm. tetanus came on at 40° after ¼ to 4 hours; at 55° after ¾ to 1¼ hours; at 70° after ¼ to ¾ hours.

These results show that tetanus may be induced by strychnin at low and at high temperatures by doses which will not cause tetanus at temperatures between. They also show that the interval between injection and the onset of tetanus grows less with higher temperatures, although there is no constant ratio.

65 (761)

On the difference in the effect of Gréhant's[1] anesthetic and of morphine-ether on the total output of urine and the composition of the urine in normal dogs.

By WM. DEB. MACNIDER.

[*From the Laboratory of Pharmacology, University of North Carolina.*]

In a recent study of the action of various diuretics in uranium nephritis,[2] it was shown that the anesthetic employed in the experiments not only influenced the output of urine, but that following the anesthetic, diuretic substances such as caffeine, theobromine, and 0.9 per cent. sodium chloride lost their diuretic value.

The following experiments are being conducted to ascertain if the previously mentioned anesthetics have any effect in reducing the output and in changing the composition of the urine in the normal dog and if the anesthetics differ from one another in their action.

When full grown dogs are given Gréhant's anesthetic in the strength usually employed there is a marked reduction in the output of urine and the animal becomes glycosuric, the percentage of glucose varying between 0.165–3.33 per cent. Acetone has so far been constantly present in the urine of these full-grown animals.

An albuminuria is induced and is accompanied by the appearance of casts, hyaline, or hyaline and granular. In one of the

[1] Gréhant's anesthetic. The animal is given ¼ c.c. per kilogram of a 4 per cent. solution of morphine. This is followed in half an hour by 10 c.c. per kilogram of the following mixture: chloroform, 50 c.c.; alcohol and water, each 500 c.c.

[2] *The Journal of Pharmacology and Experimental Therapeutics*, Vol. III, No. 4, March, 1912.

animals bile appeared in the urine on the second day after recovery from the anesthetic and persisted for two days.

The anesthesia is complete for from ten to eighteen hours. Three animals have failed to recover.

When Gréhant's anesthetic is given to full-grown dogs in half the strength usually employed the output of urine is usually but slightly diminished, the animals however become glycosuric, the percentage of glucose having varied between .101–.301 per cent. The urine does not contain acetone.

Albumen is present with hyaline or hyaline and granular casts.

The anesthesia is imperfect. Recovery is usually complete within twelve hours.

In a final series of animals, puppies were used. The age of these animals ranged from six weeks to four and a half months. Gréhant's anesthetic was given in full strength. The animals were completely anesthetized for two and a half to nine hours. The output of urine was decidedly decreased and showed both glucose and albumen. The percentage of glucose varied from .0701–.202 per cent. Acetone was present in the urine of the animal four and a half months old. All of the animals recovered.

In the experiments conducted with morphine-ether, only full-grown animals have so far been employed. The anesthetic was given in sufficient quantity to keep the animal completely anesthetized for three hours. All of the animals recovered. The recovery has been usually complete within six to eight hours.

The output of urine in the twenty-four hours following the anesthetic has been but slightly reduced, excepting in one animal that was very old. In this animal the urine was reduced from 515 c.c. on the day prior to the anesthetic to 320 c.c. in the twenty-four hour period following the anesthetic. The urine of this dog showed a fairly heavy precipitate of albumen and .104 per cent. of glucose. Acetone was not present.

The remaining animals of this series have not developed a glycosuria, and the urine has been free from acetone, albumen, and casts.

Experiments will be continued to ascertain if there exists any relation between the age of the animal and the duration of the anesthesia, and between the duration of the anesthesia and the appearance of various abnormal constituents in the urine.

66 (762)

The rate of growth in the dog.

By SUTHERLAND SIMPSON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

Observations have been made on twenty-one litters. As soon after birth as possible, when the amniotic fluid had been dried off, each individual was weighed and at the same time it was ear-marked and the sex determined. For the first three or four weeks the weights were taken daily, at the same hour (4 to 5 p.m.), and later every third day. From the figures obtained curves were plotted showing graphically the absolute weight increments and the rate of growth. The figures for the number of young, the proportion of the sexes, the birth mortality, and the body weights when born, are given in the following table.

No. of Litter.	Number Born.					Average Weight at Birth.	
	Alive.		Dead.		Total.		
	♂	♀	♂	♀		♂	♀
1	4	4	—	—	8	294	289
2	4	2	1	—	7	278	280
3	—	4	—	—	4	—	271
4	4	4	—	—	8	270	266
5	6	5	1	—	12	279	274
6	4	4	—	—	8	280	268
7	3	4	—	—	7	298	294
8	1	7	—	—	8	255	286
9	1	2	—	—	3	354	288
10	4	—	—	—	4	309	—
11	2	3	—	—	5	255	245
12	3	3	—	—	6	278	297
13	3	5	—	—	8	310	310
14	4	4	—	—	8	269	218
15	3	2	1	—	6	242	253
16	1	—	—	1	2	318	—
17	4	6	—	1	11	305	294
18	1	1	—	—	2	365	297
19	—	3	—	—	3	—	299
20	5	2	1	—	8	344	322
21	1	4	1	—	6	238	241
	58	69	5	2	134	292	279

The number of young in a litter varies from 2 to 12, families of 8 occurring most frequently. The proportion of the sexes is in favor of the female, the ratio being 100 males to 119 females.

This does not agree with the statistics for the human subject nor with Minot's results in the guinea-pig, but, of course, the number of individuals so far examined by me is too small to draw any general conclusion from. The birth mortality is highest in the males, as is also the average body weight when born.

Minot found that in male guinea-pigs, as in newborn children, there is an actual loss of weight for the first 3 or 4 days after birth. Such, however, is not the case in the dog. In almost all the litters there is some gain in 24 hours, and this is very decided at the end of the second day. There is a post-natal retardation of growth but it is of relatively short duration.

67 (763)

The influence of experimental cretinism upon nitrogenous metabolism in the sheep.

By ANDREW HUNTER.

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

The object of the investigation was to determine whether athyroidism in sheep is associated with any striking abnormality of intermediary metabolism, such as might be revealed by the nitrogen partition of the urine. The subjects were three sheep which have already been described before this Society by Simpson.[1] At the age of two months they had suffered the loss of the thyroid and internal parathyroid glands, and had subsequently developed into typical cretins. One year after the first operation the external parathyroids also had been removed. While the later condition was of course not that of uncomplicated athyroidism, symptoms referable to the loss of the parathyroids were but slightly marked. Tetany, in particular, was never observed. When the animals came under my care they were one and a half to two years old.

To furnish a basis of comparison two normal sheep, nearly four years of age, were included in the investigation. As the most convenient way of avoiding the difficulties caused by variable appetite, etc., all five animals were starved. The urine was collected as voided.

[1] Simpson, PROC. SOC. FOR EXP. BIOL. AND MEDICINE, 1911, IX, 2.

The normal animals endured fasts of five days' duration with little apparent distress or enfeeblement. Of the others two rapidly weakened, and succumbed within six days; the third was in excellent condition after seven days without food. Below is shown the ascertained distribution of nitrogen in the urine of one normal and one operated animal. The records of the others were of corresponding character.

NORMAL SHEEP—WEIGHT 42 KILOGRAMS.

Day of Starvation.	Total Nitrogen.	Per Cent. Nitrogen as							
		Urea.	Ammonia.	Creatinine.	Creatine.	Allantoin.	Uric Acid.	Purine Bases.	Undetermined.
2–3	8.04	84.4	2.3	3.8	2.0	1.8	0.3	0.4	5.0
3–4	7.64	83.6	3.2	4.2	1.8	1.7	0.4	0.4	4.7
4–5	6.40	80.3	4.3	4.2	2.5	1.5	0.5	0.5	6.2
5–6	5.78	79.8	6.3	4.9	2.5	1.7	0.4	0.5	3.9

OPERATED SHEEP—WEIGHT 15 KILOGRAMS.

1–2	3.83	84.4	1.8	3.5	1.2	2.1	0.4	0.7	5.9
2–3	6.82	85.8	1.6	2.4	2.1	1.1	0.2	0.4	6.4
3–5	4.98	84.5	2.4	2.5	3.1	1.4	0.3	0.4	5.4
5–6	2.31	76.6	2.2	2.5	5.9	1.5	0.5	0.5	10.3

Nitrogen partition in the urine of the normal sheep is seen to follow the general mammalian type, the only point worthy of special note being the comparatively small proportion of purine nitrogen excreted as allantoin. In the urine of the thyroidectomized animal the only constituents apparently affected are ammonia and creatine. The percentage of the first is lower than the normal at corresponding stages of starvation. (One only of the three operated sheep—the one least affected by fasting—showed figures similar to the controls.) The creatine rises early in the fast to a higher level than the creatinine. The significance of this "creatine crossing" is not clear. It might be supposed to be related to the susceptibility of the animals to withdrawal of food; but, curiously, in the one subject that survived a week, creatine exceeded creatinine from the very first day of observation.

Complete data will be published shortly.

68 (764)

Metabolism studies in a case of hypopituitarism, with infantilism of the Lorain type.

By DEWITT STETTEN and JACOB ROSENBLOOM.

[*From the German Hospital, New York, and the Laboratory of Biochemistry of the University of Pittsburgh, Pittsburgh, Pa.*]

This report contains the results obtained on studying certain phases of metabolism in a case of a man aged 22, who presented the classical symptoms of infantilism of the Lorain type, with symptoms definitely pointing to a benign tumor, probably a cyst of the hypophysis.[1] The arrest of the body growth and of sexual development with the moderate adiposity stamped this case as a

TABLE I. (NITROGEN PARTITION.)

Urine.

Date.	Total Nitrogen.	Urea-Nitrogen.		Ammonia-Nitrogen.		Creatinine-Nitrogen.		Uric Acid-Nitrogen.		Undetermined-Nitrogen.	
		Gm.	Per Cent. of Total Nitrogen.	Gm.	Per Cent. of Total Nitrogen.	Gm.	Per Cent. of Total Nitrogen.	Gm.	Per Cent. of Total Nitrogen.	Gm.	Per Cent. of Total Nitrogen.
6/23	13.0	—	—	—	—	—	—	—	—	—	—
6/26	16.1	13.5	83.8	0.36	2.3	0.60	3.8	0.20	1.4	1.4	8.7
6/27	16.3	13.3	81.6	0.41	2.5	0.58	3.5	0.24	1.5	1.8	11.0
6/28	15.7	12.6	80.3	0.40	2.5	0.56	3.5	0.26	1.7	1.9	12.1
6/29	15.8	12.9	81.7	0.44	2.7	0.54	3.4	0.23	1.5	1.7	10.8
6/30	15.3	12.3	80.5	0.40	2.6	0.50	3.3	0.27	1.7	1.8	11.7
7/1	15.7	12.6	80.2	0.48	3.1	0.55	3.5	0.29	1.9	1.8	11.5

TABLE II. (SULPHUR PARTITION.)

Urine.

Date.	Total Sulphur Gm.	Total Sulphate Sulphur, Gm.	Ethereal Sulphate Sulphur, Gm.	Inorganic Sulphate Sulphur, Gm.	Neutral Sulphur Gm.	Sulphate-S / Total S Per Cent.	Ethereal Sulfate-S / Total S Per Cent.	Inorg. Sulf. S / Total S Per Cent.	Neut. S, / Total S. Per Cent.
6/23	0.57	0.45	0.04	0.41	0.12	78.6	6.8	71.8	21.3
6/26	0.41	0.30	0.03	0.27	0.11	72.5	7.2	65.3	27.4
6/27	0.80	0.66	0.06	0.60	0.14	82.4	7.1	75.3	17.5
6/28	0.90	0.69	0.04	0.65	0.21	76.7	4.3	72.4	23.3
6/29	0.90	0.69	0.05	0.64	0.21	76.7	5.5	71.1	23.3
6/30	0.87	0.53	0.05	0.48	0.34	61.7	6.4	55.3	38.3
7/1	0.96	0.66	0.04	0.63	0.30	69.2	4.0	65.2	30.8

[1] At operation a cyst of the hypophysis was found.

typical example of the Froehlich typus or the so-called dystrophia adiposo-genitalis of Bartels.

The patient was placed on the high protein diet of Folin and the following tables contain the data obtained in this study. The *high undetermined nitrogen* and *neutral sulphur* of the urine, can readily be noted, while the absorption of fat and protein was normal.

TABLE III.

Summary of data pertaining to total nitrogen and fat metabolism.

Date.	Total Nitrogen.			Feces.			Fat.	
	Ingested, Grams.	Excreted, Grams.	Balance, Grams.	Fat, Grams.	Total Nitrogen.		Ingested, Grams.	Percentage of Fat Absorbed.
					Found, Grams.	Per Cent. of Total N Ingested.		
6/26	18.2	17.9	+0.3	2.2	1.8	9.9	140	98.4
6/27	18.6	18.1	+0.5	2.2	1.8	9.7	140	98.4
6/28	18.3	17.5	+0.8	2.2	1.8	9.9	140	98.4
6/29	18.1	17.9	+0.2	1.8	2.1	11.6	146	98.8
6/30	17.6	17.4	+0.2	1.8	2.1	11.9	146	98.8
7/1	17.8	17.8	0	1.8	2.1	11.9	146	98.8
Total	108.6	106.6	+2.0	12.00	11.70	—	858	—
Average	18.1	17.7+	+0.33	2.00	1.95	10.8	143	98.6

69 (765)

The relation of pancreatic extract to the sugar of the blood.

By ERNEST L. SCOTT.

[*From the Department of Physiology of Columbia University.*]

In January, 1912, I reported a preparation of pancreas which when injected intravenously into dogs that had been rendered diabetic by pancreatectomy, lowered both the output of sugar and the D/N ratio. This preparation seemed to offer a ready means of attack for several of the problems bearing on the relation of the pancreas to sugar metabolism. Possibly the simplest of these is the relation between the amount of sugar present in the blood and the abundance of the pancreatic hormone present, and this is a preliminary report of my work on this subject.

Cats were killed and their blood was collected. The protein was removed and the blood was decolorized by a modification of the phosphotungstic acid method reported by Oppler. In deter-

mining the amount of sugar Munson and Walker's "Uniform method of sugar determination" was used and gave consistent results. In order to test its efficiency the method was controlled by division of a sample of blood into two portions. In one of these the glucose was estimated directly. To the other a known amount of glucose was added and then this amount was subtracted from the total recovered, leaving a remainder which should equal the amount found in the portion estimated directly. The results of one such control are shown in Table I.

TABLE I.

Blood sample 6 (two cats). Total blood used 190.05 gm.

	a	*b*	*c*	*d*
Gm. blood in sample	47.51	47.51	47.51	47.51
Gm. glucose recovered	0.0314	0.0328	0.0501	0.0497
Gm. glucose added	———	———	0.0178	0.0178
Gm. glucose in blood	0.0314	0.0328	0.0323	0.0319
Gm. glucose per 100 gm. blood	0.0661	0.0690	0.0680	0.0671
Average for *ab* and *cd*	———	0.0676	———	0.0676

It was necessary next to determine the amount of sugar in the blood of normal cats. The results for a series of ten cats are given in Table II. It will be noticed that with the exception of the cat which gave 76 mgm.—obviously an anomalous case—the greatest variation from the average is 4 mgms. The starred numbers indicate controls such as are reported in Table I. The results are calculated to grams of glucose per hundred grams of blood.

TABLE II.

0.0676	*
0.0676	
0.0618	
0.0760	
0.0653	
0.0628	
0.0627	
0.0646	*
0.0646	
0.0691	*
Average	0.0662

TABLE III.

	0.1026
	0.0783
	0.0719
	0.0811
	0.0692
Average	0.0806

The extracts of the pancreas that were used in the following experiments were prepared as reported previously, except that the

temperature was at all times kept below 50° C. instead of being allowed to go to 65° as before. So far five cats have been injected with the extract, the first three with two injections each and the others with one each. All injections were made beneath the skin of the back. The results are shown in Table III and are calculated to grams of glucose per hundred grams of blood as in Table I. The average for the injected cats is over 21 per cent. above that for the normal ones, and moveover the amount of sugar is greater in each injected cat than for any normal animal except the one that gave 76 mgm. This increase is very surprising and of peculiar interest. At present I am not willing to venture any explanation. There are however several possibilities which are amenable to experiment and I hope that further work will throw some light on it. In any case it would seem to put in grave doubt the idea that the pancreatic hormone always tends to increase the storage of glycogen in the liver at the expense of sugar in the blood.

There are a number of factors entering into the experiments so far performed that might cause the individual variations in the experimental results. Some of the more probable are: the length of time intervening between the injection and the death of the animal; the amount of extract injected; the age of the extract; the number of injections and the time intervening between them, etc. At present I am trying to find some of the optimum conditions.

70 (766)

On some blood pressor substances and adrenal separations in experimental immunity.

By **J. P. ATKINSON** and **C. B. FITZPATRICK.**

One of us in a work[1] on "The Preparation of Diphtheria Antitoxin" endeavored to demonstrate by charts of the systemic reaction following injections of cultures of the bacillus of diphtheria and its toxin that the real crux of the process of immunization was to determine when to re-inject. This question is still unsettled; in short, of two animals treated the same, upon being re-injected the one, which may apparently be the better prepared, dies and the other recovers.

[1] Fitzpatrick, *N. Y. Medical Journal*, April 27, 1895.

This aspect of immunization is very well brought out in the following example.

Römer and Joseph[1] re-infected two tuberculous sheep with a culture of which 1 mg. per 10 kg. killed healthy sheep in one month. One of these sheep was re-infected with 1 mg. per 10 kg. ten months after a previous inoculation with a .2 mg. per 10 kg. and 15 months after a first injection of .1 mg. per 10 kg. This animal died in 48 hours. The other one of these sheep was re-infected with the same dose (1 mg. per 10 kg.) ten months after previous injection of .2 mg. per 10 kg. This animal responded with an intense reaction. The reaction was followed by a return to health.

We have shown in studies read before this society that the blood of tuberculous animals especially when about to die of tuberculosis contains a depressor substance. The use of this blood in conjunction with tuberculin was likewise shown to give an effective immunity against fatal infection with the *B. tuberculosis* in one case, and in some others the fatal ending was delayed beyond the controls. It was also demonstrated that the injection of this serum shortly after the injection with the tuberculin rapidly caused death. We have also found depressor substances to be present in the blood serum of other diseases.[2]

The first part of our present report consists of results of the injection of blood serum obtained from animals recovering from inoculations of *B. tuberculosis* and the possible application of these observations to the practical therapy of infection and intoxication.

Protocol[3] April 19, 1912.—A 14 lb. female dog, sensitized April 18, 1912, with ¾ c.c. of crude tuberculin.

8 c.c. of the serum of a calf recovering from repeated doses of culture of *B. tuberculosis* (human type), when injected into the femoral vein of this dog gave a decided rise in blood pressure. This rise was preceded by a very slight depression. This dose was repeated in six minutes with a like result. Five minutes later the same dose was injected and gave no response.

[1] "Die Tuberculöse Re-infection," *Beiträge zur Klinik der Tuberculose*, 1910, XVII, pages 330–333.

[2] Atkinson & Fitzpatrick, PROC. SOC. EXP. BIOLOGY AND MEDICINE, 1910, VII, pp. 77–79; *ibid.*, pp. 104–107; *ibid.*, VIII, pp. 24–28; *ibid.*, pp. 49–51, 1912.

[3] 3 c.c. normal calf serum gave no response when injected into the femoral vein of dog sensitized with tuberculin.

Protocol July 13, 1912.—7 c.c. of serum of same calf, bled June 21, 1913, gave a fair rise. The extract in saline solution of the adrenal gland of this calf gave a very high rise, 1 /16 c.c. being sufficient to cause a very marked straight rise.[1] 7 c.c. of tuberculous (bovine type) dog serum gave no response. This dog was killed and was probably dying according to the autopsy findings.

Protocol August 28, 1912.—8 c.c. of serum from a dog, recovering according to autopsy findings from tuberculosis (bovine type) gave a fine rise. Repeated with same results three times.

Protocol January 5, 1910.—2 c.c. of the serum from a rabbit with tuberculosis, bovine type, gave a fair, sustained rise.

Protocol April 6, 1912.—8 c.c. of serum from a dog recovering from tuberculosis, bovine type, gave a marked rise. Repeated three times.

8 c.c. of this serum, injected into a dog sensitized with tuberculin, gave a marked rise, when added to 10 drops of tuberculin No. 5, which ordinarily caused a fall.

Further observations of the occurrence of pressor substances were made as follows in animals about to die from hydrophobia.[2]

Protocol 16.—7 c.c. of serum obtained from a rabbit (June 21, 1912), sick with hydrophobia, gave a marked rise. Femoral injection.

7 c.c. of serum from a goat with hydrophobia gave a slight rise. Femoral injection.

Protocol March 17, 1911.—7 c.c. of serum from a dog with hydrophobia, gave a slight depression.

Protocol April 8, 1911.—5 c.c. of serum of rabid dog, bled April 8, 1911, gave marked rise. Repeated once.

4 c.c. of serum from collie with hydrophobia also gave a marked rise.

7 c.c. from a dog with dumb rabies gave a rise, bled April 5, 1911.

7 c.c. from a dog with rabies (March 17, 1911) gave a rise.

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[1] The extract of the adrenal glands of rabbits which died of tuberculosis showed little or no pressor substance.

[2] We found the pressor substance present in the serum of animals (dog and goat) suffering from street rabies, bled just before their death. These animals with the exception of the rabbit were naturally infected.

6 c.c. from a collie with rabies gave a marked rise, bled March 24, 1911.

Protocol of March 31, 1911.—3½ c.c. of serum from collie with hydrophobia gave a very marked rise. Repeated three times.

6 c.c. of serum from fox terrier, with hydrophobia, tested March 17, 1911, gave a very marked rise. Repeated twice.

Protocol November 9, 1910.—1½ c.c. of serum from a dog with hydrophobia, bled November 3, 1910, gave a slight rise; 5 c.c. gave a fair rise.

Protocol January 6, 1912.—7 c.c. of serum from a hydrophobia dog, of January 2, 1912, gave a fair rise.

The apparent contradiction between the recovering tuberculous animal and the dying hydrophobia animal may be explained by our previously reported observations,[1] where the continued injection of a depressor substance instead of causing death led to an actual increase in blood pressure.[2]

It would appear that animals possessing these pressor substances do not die as readily from infection or intoxication as those which do not have these pressor substances. It appears to us that we may have here a general law for many, if not all infections and intoxications.

We have found two substances in adrenal gland preparations.

A substance causing increased pressure and a depressor substance.

In commercial preparations we found these two substances to be present.

Separations were made by alcohol, ether, chloroform and normal saline solution.

In saline extracts of fresh glands, the substance causing increased pressure was present alone or so greatly in excess as to dominate the reaction.

In the adrenals of certain diseased animals (tuberculosis,

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[1] PROC. SOC. EXP. BIOLOGY AND MEDICINE, 1912, IX, pp. 49–51.

[2] It would also be of interest to study the origin of these pressor substances in normal and diseased tissues. Protocol of January 16, 1913, for example shows that the injection of 5 c.c. normal dog brain extract, gave no response, while the same doses of tuberculous and hydrophobia brain extracts gave a slight rise. These extracts were made with saline solution.

vaccinia, septic pneumonia, etc.) the substance causing increased pressure and the depressor substance were found.

The organs which possess pressor substances are apparently important organs of immunization. The organs with the depressor substances are possibly eliminating or fixing the split-products or poisons arising from the disease. The adrenals are probably simply one of a number of organs, which possess pressor producing tissues, and which when acting altogether in the whole living organism produce the general pressor substances or immuno-pressor substances. In vivo these pressor substances are probably present in every tissue and form part of the factors which overcome infection. They may be the first step or a very important step in overcoming infection.

These results apparently furnish an indicator as to when and how to re-inject animals already afflicted artificially or naturally with an infection or intoxication, with the purpose of immunizing and healing the diseased animals. That the rôle of these pressor substances, present in experimental immunity is important, we do not doubt. The use of an immunizing dose which is just sufficient to cause their production may be what these observations indicate.

71 (767)

Preliminary communication on a complement deviation reaction exhibited in pregnancy.

By G. H. A. CLOWES, FRANCIS C. GOLDSBOROUGH, and F. WEST.

[*From the Laboratory of Biological Chemistry, State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

In a series of twenty-five normal pregnant women at term in which syphilis could be excluded the blood of the mother was taken from the vein during labor and the blood of the infant from the cord at the time of delivery. The sera after separation from the clots were frozen and allowed to remain in the icebox 48 hours before being employed. A series of complement deviation tests were carried out, using both unheated and heated sera of mothers

and infants in conjunction with a large variety of antigens with the following results. The unheated mothers' sera invariably contained antibodies capable of causing a well-marked deviation of the complement when used in conjunction with an antigen obtained by extracting human blood clots with alcohol. The unheated infants' sera tested under the same conditions invariably showed completely negative results. A large variety of alcoholic extracts of tissues used as antigens gave similar although somewhat less marked deviation with unheated mothers' sera and absolutely negative results with unheated infants' sera. The deviating body concerned in this reaction is destroyed by heating for ½ hour at 58° C. The mother's sera tested after heating were negative to the antigens enumerated above, and those of the infants either negative or very slightly positive, but exhibiting on the whole a somewhat greater capacity to deviate than that possessed by the heated mothers' sera. The deviating capacity of unheated mothers' serum varies greatly, certain cases exhibiting a complete deviation only when employed in concentrations as high as .05 to .075 c.c. of serum, others giving a complete deviation when amounts as small as .001 c.c. of serum were employed. It is important to note that the antibodies in question occasionally fail to make their appearance until after the serum has been frozen for two or three days as indicated above. Similar non-specific immune bodies destroyed by heating at 58° C. have been observed in cancer and other pathological conditions,[1] and to a certain extent in supposedly normal individuals. The entire absence of these bodies in the blood of newborn infants and their invariable occurrence in the blood of pregnant women at term indicates that in this case at least they probably bear some relation to the reaction of the body against detached fetal cells or proteid or enzymatic bodies of fetal origin. The occurrence of this reaction to a marked extent in cancer, particularly in those cases in which tumors are absorbing under treatment, lends further support to this point of view.

[1] Clowes, *Zeitschrift f. Krebsforschung*, 1912, Vol. XII, page 421.

72 (768)

On the antitryptic reaction exhibited in pregnancy.

By G. H. A. CLOWES and FRANCIS C. GOLDSBOROUGH.

[*From the Laboratory of Biological Chemistry, State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

The antitryptic index has been determined in a series of twenty-five pregnant women and their infants. The blood was taken during labor from the vein of the mother, and from the cord of the infant at birth, was allowed to clot and the separated serum employed for tests which were carried out by means of the Oswald viscosimeter, making use of a method previously described.[1] The antitryptic index of the mother's serum was found to range from 1.5 to 2.5, averaging about 2, whilst that of the infants was found to range from .9 to 1.2, averaging slightly over 1. The antitryptic index of a series of cancer cases[1] previously reported shows a range of variation from 1.2 to 3.5 and gives an average over 2. It will thus be seen that the blood of this series of twenty-five infants appears to be practically normal. On the other hand the blood of the mothers contains an extremely high percentage of antibodies to trypsin, averaging over twice the normal, a characteristic also exhibited by cancer blood. This antitryptic reaction is destroyed by heating the serum to 60° for ½ hour in which respect it resembles the complement deviating reaction referred to in the previous paper from which it may be concluded that whilst these two reactions do not follow absolutely parallel lines in a quantitative sense, they probably have a common origin. The fact that reactions of this type invariably occur in pregnant women and in cancer cases and are entirely absent in infants lends support to the theory that they result from a reaction of the body against enzymes or other products of fetal origin in pregnancy, and similar products derived from the tumor in cancer.

[1] Clowes, *Zeitschrift f. Krebsforschung*, Vol. XI, page 146.

73 (769)

On a new factor in passive anaphylaxis.

By RICHARD WEIL.

[*From the Department of Experimental Therapeutics, Cornell University Medical School, New York City.*]

After active sensitization guinea-pigs remain hyper-sensitive for at least three years—probably for life. After passive sensitization with homologous serum, *i. e.*, with the blood of an immune guinea-pig, they retain the sensitive condition so conferred for a period of 60 or 70 days at least. After passive sensitization with heterologous serum, *i. e.*, with the blood of immune rabbits, they lose the sensitive condition in 10 days or less. This rapid loss has never been explained. It might theoretically be due to the development by the injected guinea-pig of immune substances directed against the introduced rabbit serum, which would then neutralize or destroy the rabbit anti-bodies, on the presence of which sensitization depends. This theory would explain the fact that the injected guinea-pigs retain their sensitiveness for about ten days, which would correspond to the time necessary to develop anti-bodies.

In order to test this idea, normal guinea-pigs were given a subcutaneous injection of normal rabbit serum. After eight days they were sensitized with a very large dose of the serum of a rabbit immunized against horse serum, given intra-peritoneally. Two days later, when tested by an intravenous injection of horse serum, they failed to manifest any symptom of anaphylaxis. The controls, although sensitized with a much smaller dose of immune rabbit serum, and intoxicated with about one fiftieth of the amount of horse serum used in the previous series, succumbed without exception.

The refractory state towards passive sensitization, as thus induced, may occasionally be demonstrated within three days of the first injection.

Partial immunization of guinea-pigs against rabbit antibodies can sometimes be obtained by the previous injection of sheep serum. It is, therefore, not strictly specific.

74 (770)

Hypertrophy of the thryoid gland. Revision of experiments made 25 years ago.

By WILLIAM STEWART HALSTED.

For some years I have thought that the hyperplasia of the remaining thryoid tissue which has followed excision of a portion of the thyroid gland might be due to infection of the wound, and was, in most cases, not a compensatory hypertrophy; and for the following reasons:

1. In 1888 I found that hyperplasia of the thyroid glands of dogs occurred after the injection of several c.c. of a bouillon culture of *Staphylococcus aureus* into the peritoneal cavity, and also when a mild form of peritonitis had been produced in these animals—a peritonitis which was not rapidly fatal.

2. Experiments conducted in 1906 and 7 in the Hunterian laboratory seemed to prove that for the successful transplantation of a parathyroid glandule, a considerable deficiency must be created.

3. Have observed that symptoms of hyperthyroidism and even exophthalmic goiter may develop promptly after tonsillitis, appendicitis, pneumonia, typhoid fever and other infections.

4. Twice in the course of the past five years I have had the opportunity to examine the remaining lobe of the thyroid gland after excision of the other in dogs whose wounds had healed throughout without suppuration and have noted that there was no hyperplasia of the former.

5. The inconstant results obtained by other experimenters.

Last October I proposed to Dr. Hunnicutt, my assistant the Hunterian laboratory, that he undertake a series of experiments with view to determining the matter definitely. Observing aseptic precautions in the strictest manner Dr. Hunnicutt has made a large number of experiments and we are able to report that in the nine dogs whose thyroids thus far have been examined there has been not the slightest evidence of hyperplasia in a single instance. The average time allowed to elapse between the removal of the first and second lobes was 55 days, the shortest

interval being 30 and the longest 81 days. In one of the dogs there was a superficial stitch infection for a few days, otherwise there was in no case the slightest evidence of suppuration.

From a restudy of the report of my experiments on extirpation of the thyroid gland made in 1888, I find that, for the major part of the experiments, the wounds of the dogs were left open, and that after 22 days, with 3 exceptions (Nos. 105, 126 and 127) there was hypertrophy, macroscopic and microscopic, of the remaining gland in the animals whose wounds were permitted to heal by granulation, whereas when the wound healed absolutely per primam the hyperplasia of the remaining thyroid tissue did not develop except in dogs which died from some intercurrent disease such as pneumonia or distemper.

Of the three exceptions referred to the tip only of one thyroid gland was ligated in two (nos. 126 and 127) and no tissue removed at the operation. 132 days later, examination of the thyroid lobe revealed no hyperplasia, except perhaps to a very slight and questionable degree in one of the sections of the lobe of one of these dogs. The absence of hypertrophy in dogs 126 and 127 after 132 days may possibly have been due to the long interval between the first and second operations. The hyperplasic picture may, possibly, have been present and vanished, although from observations of 25 years ago we know that extreme hyperplasia may persist for 104 days and we have no absolute proof from them that having been once established it disappears. From a study of the elaborate and important work of David Marine and Marine and Lenhart I am quite convinced that the return to normal is to be expected. In one of Dr. Hunnicutt's dogs the lobe first removed showed marked hyperplasia, whereas 87 days later the second lobe presented the normal histological appearance.

In the remaining case (No. 105) the veins at the upper poles of both lobes were ligated and unnecessary manipulation carefully avoided. On the 51st day there was no evidence of hyperplasia.

That there is such a thing as true compensatory hyperplasia is proved, I think, by my experiments in transplantation of the parathyroid glandules. Thus when both thyroid lobes have been

[1] Johns Hopkins Hospital Reports, Vol. I, Table IV.

removed and only a film of thyroid transplanted with one parathyroid gland, this film hypertrophies enormously and on microscopical examination displays the typical picture of hyperplasia. In one instance the graft was examined 15 months after operation.[1] It would be interesting to determine the amount of deficiency which it is necessary to create for the successful transplantation of the thyroid gland; also whether hyperplasia necessarily ensues when the transplant lives. In other words will a thyroid graft always fail to take unless the deficiency created is so great that hyperplasia must develop?

Should it become a definitely established fact that hyperplasia may be produced by infection, not only are a number of things explained which otherwise seemed inexplicable, but new lines of investigation suggested.

75 (771)

Partial occlusion of the thoracic and abdominal aortas by bands of fresh aorta and of fascia lata

By W. S. HALSTED, M.D.

Ligation of the human abdominal aorta has been made 19 or 20 times and always with fatal result.

Dubois, Assalini, Bujalsky, Pirigoff, Cooper, Keen and perhaps others attempted to occlude the abdominal aorta gradually by means of cleverly devised instruments which, carrying snares of silk, metal or catgut might be tightened or loosened at will. The instruments traversed the abdominal wall and hence infection was a complication common to all of the methods and defeated the plans of the operators.

In 1904 assisted by Dr. W. F. M. Sowers, I began a series of experiments on dogs in the hope of finding a safe method of occluding the aorta and curing aortic aneurysm. Bands of silver and aluminum curled about the aorta by an instrument constructed for this purpose were rolled tighter by the fingers until the desired degree of occlusion of this vessel was obtained. The abdominal wounds were closed with the expectation that they would have to be reopened one or more times for the purpose of

[1] *Journal of Experimental Medicine*, 1912, Vol. XV, Plate 30, Fig. 2.

progressively occluding the lumen of the artery. But in the course of our experiments we had opportunities to make trial in the human subject of partially occluding bands on other arteries (innominate, subclavian, carotid, femoral, popliteal) whose blood streams in some instances it seemed unsafe to cut off suddenly and completely, and found that incomplete occlusion of an artery sufficed to cure the aneurysm, possibly quite as surely as might have been expected of total occlusion. Hence, tentatively, I abandoned the idea of progressive closure of the aorta, determining, instead, to obliterate the lumen of this vessel, in the attempt to cure its aneurysm, to an extent which we had found quite safe in the dog.

I have applied an aluminum band to the human aorta four times—twice in one subject—and twice with promising results so far as the cure of the aneurysm is concerned. But the experimental work on animals had led me to expect that ultimately the metal bands must cut through the artery, because in cases observed for 7 months or less the wall of the aorta had become atrophied to an extreme degree and there was no adhesion between the infolded, attenuated surfaces. That my fears were well founded was proved by an experience in Europe, about 18 months ago. The patient was an aged woman with dilated and badly functioning heart. The large aortic aneurysm was well located for the placing of a band which was applied just below the renal vessels. Within a few days the aneurysm which before operation was distinctly visible from the seats of the operating amphitheater was barely discernible at the bedside, and at the end of six weeks had disappeared so completely that the patient was discharged apparently cured. But, walking out of the door of the hospital she was seized with a pain and returned to her bed. The following morning she died from hemorrhage. The aorta had ruptured at the side of the band, but the aneurysm was found to be nearly cured.

Stimulated by the results in this case to further experimentation it occurred to me to test the behavior of cuffs and spiral strips of the fresh aorta of one dog when wound about the aorta of another. So on the 29th of April, 1912, I operated upon two dogs, partially occluding the aorta of one of them with a spiral aortic band and of the other with a cuff cut from the same vessel.

Strips of aorta were employed rather than of fascia lata, for example, because I hoped that the elastic tissue, in case it did not live, might, at least, serve its purpose for a time sufficient to cure an aneurysm.

At the end of two months one of the dogs was killed and I was pleased to find that the cuff which had been used in this experiment was apparently organized and had not stretched to any appreciable extent. Above the cuff the aortic pulse was forcible, but below the constriction it was very feeble, though countable and accompanied by a thrill.

The other dog operated upon at the same time and in the same manner, except that a spiral band of aorta instead of a cuff had been employed died (cause of death unascertained) about three weeks after the operation. In this instance the aorta had been almost completely occluded by the spiral aortic strip. The weldlike band had not stretched and seemed to be organized. The aorta on being split longitudinally was seen to be greatly and characteristically infolded and almost occluded at the site of the band. Sections of the specimens indicate that the elastic coats of the bands as well of the included artery are intact. During the present winter I have made many similar experiments employing various fresh tissues for the bands with most encouraging results. We have learned, however, that whereas the spiral bands seem to be perfectly safe there is danger in the employment of the cuffs. In two instances of twelve or more experiments one of the mattrass sutures taken to hold the flaps of the cuffs together cut through one side and thus being brought in contact with the aortic wall cut a minute hole in the vessel through which the animal bled to death. Such an accident can hardly happen with the employment of the spiral strip, for not only is the strain on the stitches very slight when this form of band is used, but even if it were so great that a thread might cut through the spiral at any point, it could hardly be brought to bear upon the aorta in such way as to wear into its wall.

The spiral strip is wound twice about the aorta, and when one or perhaps two stitches have been taken at one end to hold the contiguous edges of the spiral together at this point, the other end of the strip is pulled upon until the aorta is occluded to the desired

amount, and then two additional stitches are taken to maintain this degree of constriction.[1]

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

Second meeting.

San Francisco, California, February 1, 1913.

76 (772)

Studies on the nature of biological specificity.

By FREDERICK P. GAY and T. BRAILSFORD ROBERTSON.

[*From the Hearst Laboratory of Pathology and Bacteriology and the Rudolph Spreckels Physiological Laboratory of the University of California.*]

Our previous immunological studies[2] with the split products of casein and a compound of casein with protamin, which were undertaken for the purpose of gaining some insight as to the nature of biological specificity have apparently been fruitless in so far as the main point at issue was concerned, with one exception. In our comparative studies of the antigenic properties of split paranuclein with paranuclein synthesized by the reversible action of pepsin from the products of peptic digestion, we were apparently able to demonstrate the genesis of an antigenic property.

The present study deals with the investigation of the antigenic

[1] About 3 weeks ago I received from Dr. Francesco Nassetti a reprint of a paper by him entitled "Avvolgimento di vasi Sanguigni con lembi liberi di aponeurosi," and published April 26, 1912, in the *Atti della R. Academia dei Fisiocritici in Siena.* Dr. Nassetti's experiments were made in the Istituto di Patalogia Speciale Chirurgica della R. Università di Siena, which is under the direction of Prof. A. Salomoni. His first experiment (a band of fascia about the carotid artery) antedates mine by 56 days, and his article appeared about three months before the publication by me of a brief account of my first experiments with spiral strips of aorta (*Johns Hopkins Hospital Bulletin*, July, 1912, p. 217). My first experiment (Apr. 29, 1912) was made 3 days after the publication of Nassetti's report. Hence the credit for the idea of wrapping blood vessels with bands of fresh tissue belongs, I am happy to say, to Italy, the country of the famous surgeon, Luigi Porta, who was, I think, the first to attempt the partial occlusion of an artery (the aorta). I have the impression that Porta used for this purpose a strip of diachylon plaster.

[2] Gay, F. P., and Robertson, T. B., *Jour. Exper. Med.*, 1912, XVI, 470; *Jour. Biol. Chem.*, 1912, XII, 233; *Jour. Exper. Med.*, 1912, XVI, 479.

properties of our second compound of casein with a non-antigenic protein, globin caseinate. We find that globin is non-antigenic and highly toxic, producing in guinea-pigs the typical symptoms and lesions of anaphylaxis. When compounded with casein, it still remains slightly toxic. An anti-serum derived by repeated injections of rabbits with globin caseinate contains fixation bodies for casein, globin caseinate, and, curiously enough, for globin, although globin alone does not produce such antibodies. By absorption experiments it may be shown that the antibodies in anti-globin caseinate serum are two in number, one for casein and one for globin. Thus it appears that the change in globin brought about by this combination with casein renders it antigenic.

A further study of similar and of more complex compounded proteins should give further insight as to the nature of specificity.

77 (773)

On the nature of oöcytin; the fertilizing and cytolyzing substance in mammalian blood-sera. (Preliminary communication.)

By T. Brailsford Robertson.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

I have elsewhere shown[1] that the agent in ox-serum which brings about the formation of fertilization-membranes in sea-urchin eggs[2] can be isolated in an impure condition by a process consisting, essentially, in precipitating the substance by barium chloride, re-solution of this precipitate in dilute acid, removal of the excess of barium by excess of sodium sulphate, and re-precipitation by acetone.

The preparations thus obtained were found to be contaminated by a considerable proportion of sodium sulphate, precipitated together with the fertilizing agent by the acetone. They also

[1] T. Brailsford Robertson, *Journal of Biol. Chem.*, XI, 1912, p. 339; XII, 1912, p. 163; *Arch. f. Entwicklungsmech.*, XXXV, 1912, p. 64.

[2] J. Loeb, *Arch. f. d. ges. Physiol.*, 118 (1907), p. 36; 122 (1908), p. 96; 124 (1908), p. 37; "Die chemische Entwicklungserregung des tierischen Eies," Berlin, 1909, p. 185.

contained appreciable traces of phosphates. I have obtained a much purer preparation by a process consisting, essentially, in precipitation from the serum by acetone, extraction of the precipitate with hot N/10 HCl, exactly neutralizing the extract with $Ba(OH)_2$, re-dissolving the precipitate in N/10 H_2SO_4 and re-precipitating it with acetone. The yield from a liter of ox-serum lies between 10 and 40 milligrams.

The substance which is thus obtained is soluble in dilute acids, alkalies and salt-solutions. It is much more rapidly dissolved when these solvents are hot. Its solutions are not coagulated by boiling. It is thrown out of solution by chlorides of the alkaline earths. It yields the Millon, Acree-Rosenheim and xanthoproteic tests for protein. One part of the substance rubbed up in 512,000 parts of sea-water caused membrane-formation in 80 per cent. of *Strongylocentrotus purpuratus* eggs which had previously been sensitized by 4 minute's immersion in 3/8 m $SrCl_2$.

The active substance, therefore, is either a protein or a peptone, or else, by the above methods of preparation, is precipitated together with a protein or peptone.

I find that Witte's "peptone" contains the membrane-forming substance, since one part of Witte's "peptone" dissolved in 16,000 parts of sea-water caused membrane-formation in 32 per cent. of sensitized *purpuratus* eggs. Hence the membrane-form-agent is digested either with difficulty or not at all by pepsin.

I find that the addition of 0.08 per cent. of lecithin or cholesterin to rabbit serum does not affect, either qualitatively or quantitatively, the membrane-forming and cytolyzing action of the serum. It would appear very unlikely, therefore, that the active substance is a lipoid.

78 (774)

On the influence of lecithin upon the development of sea-urchin embryos. (Preliminary communication.)

By T. BRAILSFORD ROBERTSON.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

When the eggs of *Strongylocentrotus purpuratus* are fertilized by sperm in a mixture of 50 c.c. of sea-water and 5 c.c. of a 1.7

per cent. suspension of lecithin in m/2 NaCl, the inner and outer fertilization-membranes are slowly dissolved by the lecithin, with the result that in the course of some six hours the cleavage-cells which have formed fall apart and ultimately disintegrate.

The eggs of *Strongylocentrotus purpuratus* female were divided into two portions. Both portions were placed in sea-water and fertilized with sperm. After 24 hours both lots of eggs had developed into free-swimming blastulæ. One portion was now transferred to a mixture of 50 c.c. of sea-water and 5 c.c. of a 1.7 per cent. suspension of lecithin in m/2 NaCl for a period of 24 hours and then returned to normal sea-water. The other portion was left in normal sea-water. The following table shows the relative development of the two portions:

Time After Fertilization.	Portion 1 (Controls).	Portion 2.
1 day.....	Blastulæ.	Blastulæ. (These were now transferred to the lecithin mixture for 24 hours.)
2 days.....	Gastrulæ.	Blastulæ. (These were now transferred to normal sea-water.)
3 days.....	Gastrulæ.	Blastulæ.
4 days.....	Gastrulæ and early plutei.	Blastulæ.
5 days.....	Fully developed plutei.	Blastulæ and 25 per cent. gastrulæ.
6 days.....	Advanced plutei.	Early gastrulæ with narrow, unbranched intestine and large, clear body-cavity.
7 days.....	Advanced plutei.	Unchanged.
8 days.....	Advanced plutei.	The gastrulæ are now retrograding; the intestine has almost disappeared.
9 days.....	Unchanged.	Unchanged.

Bacterial invasion terminated the experiment after the 9th day. It is evident that the immersion of blastulæ for 24 hours in a 0.15 per cent solution of lecithin enormously retards their development.

If *purpuratus* eggs are fertilized by sperm in more dilute solutions of lecithin in sea-water (0.003 per cent. to 0.015 per cent.) the fertilization-membranes are not dissolved sufficiently rapidly to affect the development. In these solutions development is not appreciably retarded until the blastula stage is reached. Thereafter development is very markedly retarded, and the retardation is greater the greater the concentration of the lecithin. The

embryos are not injured by the lecithin, however, as they will ultimately develop to normal plutei if left in these solutions for a sufficient time.

If cholesterin, suspended in a mixture of m/100 sodium oleate and m/2 NaCl be mixed with the lecithin in equal proportions the retarding action of the lecithin upon the development of *purpuratus* eggs is almost completely neutralized. The slight retardation which is observed in these mixtures may be due to the sodium oleate which is employed to keep the cholesterin in suspension, since sodium oleate is very toxic for sea-urchin eggs and embryos.

Cholesterin itself, when added to sea-water, has no influence upon the rate of development of the eggs. The emulsions of cholesterin are, however, coagulated by the salts in sea-water and the cholesterin is completely thrown out of suspension in the form of coarse flocculi.

79 (775)

On acid agglutination as a method of differentiation of bacteria.

By **H. J. SEARS.**

[*Division of Bacteriology, Department of Medicine, Stanford University.*]

Michaelis and several other workers following him have claimed for the phenomenon of acid agglutination a specificity comparable with that of specific serum agglutination. The reaction is specific, they say, in that optimum agglutination is produced in suspensions of bacteria of a single species by a definite concentration of hydrogen ions, irrespective of the acid used, and in that this concentration is, in general, different for different species. Their method has been to prepare solutions of definite hydrogen ion concentrations by using mixtures of a weak acid with its sodium salt, the concentrations being calculated from the formula

$$C_H = k \cdot \frac{C_{acid}}{C_{salt}},$$

where k represents the dissociation constant of the acid used. The differences between the constants obtained in this way for

the different species are especially marked in the case of the typhoid-colon group. Schidorsky and Reim claim to have had considerable success in the practical diagnosis of typhoid using this method.

Jaffé, working with a number of strains in the typhoid-colon group, obtained constants differing somewhat from those of Michaelis, and observed also several strains of each species which showed markedly different behavior toward the reagents from that of the general average.

In my investigations I made use of six strains of *B. typhosus* and four of *B. coli*. In two sets of experiments, using in one case, acetic acid and sodium acetate, and in the other, lactic acid and sodium lactate, the behavior of five strains of *B. typhosus* was shown to be the same toward both of these reagents. Three of the strains regularly failed to agglutinate at all even when concentrations of hydrogen ion varying between .7 and 900 $\times$ 10^{-5} were used. The other two strains agglutinated about equally in all the concentrations between 14 and 200 $\times$ 10^{-5}. Variations in the concentrations of salt present between N/40 and N/200 made no noticeable difference.

Using acetic and lactic acids without the presence of the salt, the same three strains failed to agglutinate, the other two, however, showing agglutination between the values of 60 and 300 $\times$ 10^{-5}. The hydrogen ion concentrations in these cases were calculated from the formula

$$C_H = \sqrt{k \cdot C_{acid}}.$$

Comparing these acids with the strong acid HCl, which can be assumed to be completely dissociated in dilutions above N/100, it was found that the three strains which showed resistance toward the weaker acids were partially agglutinated by values of C_H of from 50 to 500 $\times$ 10^{-5} while the other two were completely agglutinated by these concentrations. A sixth strain also showed partial agglutination by the same values.

The conclusions to be drawn from these results would seem to be, first, that the hydrogen ion concentrations necessary to bring about agglutination in suspensions of typhoid bacilli may differ considerably with the way in which these concentrations are ob-

tained; second, that equal agglutination is obtained in wide ranges of concentrations; and third, that these facts together with the existence of atypical strains of both the *B. typhosus* and the *B. coli* make the differentiation of these two species by this method extremely uncertain.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-third meeting.

Physiological Laboratory, University and Bellevue Hospital Medical College, April 16, 1913. President Ewing in the chair.

80 (776)

Heliotropism and galvanotropism in Euglena.

By **Frank W. Bancroft.**

[*From the Department of Experimental Biology of the Rockefeller Institute for Medical Research.*]

Hitherto positively heliotropic *Euglenæ* have always been found to give the motor reaction when suddenly shaded, and not when suddenly illuminated. Conversely negatively heliotropic individuals were found to react only to sudden illumination and not to sudden shading. Upon this association Jennings has based his theory according to which heliotropic orientation in *Euglena* is by "trial and error."

It has been found, however, that it is possible to obtain positively heliotropic *Euglenæ* which give the motor reaction when suddenly illuminated and not when suddenly shaded. It is also possible to obtain at will negatively heliotropic organisms which give motor reactions when suddenly shaded, and not when suddenly illuminated. Under certain conditions the motor reaction to shading is given by *Euglenæ* in which no heliotropism can be demonstrated at that light intensity. Under other conditions distinct negative heliotropism is obtained with a light intensity which does not bring about any motor reactions when allowed to shine suddenly on the organisms, or when they are suddenly shaded. These facts show that heliotropic orientation in *Euglena* does not depend upon the motor reactions, but upon a separate mechanism.

Galvanotropism, which has so far eluded observation in *Euglena* was obtained by using culture media containing citric acid. With this material it was found that the method of orientation is identical in both galvanotropism and heliotropism. Consequently the orientation to light is as direct as the locomotor mechanism of *Euglena* permits, and does not take place by "trial and error."

81 (777)

The fat content, morphology and length of life of cells growing in diluted blood plasma.

By ROBERT A. LAMBERT.

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University.*]

Cells growing in unmodified blood plasma (Harrison's method) exhibit regularly an accumulation of fat droplets in their cytoplasm. In the case of the cells of the chick embryo this fat accumulation is quite marked after 24 to 48 hours, and reaches a maximum after five to seven days, at which time the cells are distended with fat droplets.

The experiments herewith reported were planned to determine the effect of a reduction in the fat content of the culture medium, brought about by dilution of the plasma, on the accumulation of fat by the cells. In the course of the experiments the influence of dilution on the length of life and morphology of the cells was also observed.

One part of plasma added to twenty or twenty-five parts of Ringer's solution forms a medium which coagulates satisfactorily in hanging drops. Studies were made with dilutions of 1 : 2, 1 : 5, 1 : 10, 1 : 15, and 1 : 20 of pigeon plasma in Ringer's solution, containing 0.9 per cent. NaCl. Pieces of chick embryo heart were used for cultivation. The tissue was finely divided into pieces of suitable size for cultures, which were washed in Ringer's solution for a half hour before using. Cultures from the various series were fixed in formalin at the end of two, three and four days and stained with hematoxylin and Sudan III.

The results may be briefly summarized:

In plasma diluted 1 : 2 the cells live practically as long (5 to 10 days without transfer), as in pure plasma, and show a similar accumulation of fat.

In a 1 : 5 dilution the fat content is slightly diminished; there is little or no effect on the length of life or on the morphology of the cells.

In a 1 : 10 dilution the fat content of the cells is definitely reduced; the cells appear smaller and are stained more deeply, and the duration of life is shortened (3 to 5 days).

In higher dilutions (1 : 15 and 1 : 20) the accumulation of fat is reduced to a minimum, a majority of the cells showing at the end of two days a complete absence of fat granules. The cells which do contain fat show, as a rule, a single rather large droplet instead of a number of small droplets, as in the controls in undiluted plasma. Two to three days represents, as a rule, the limit of activity of the preparation. When stained the cells in cultures of high dilution exhibit a rather striking contrast to those in pure plasma cultures: they are smaller, more irregular in shape, and take a deeper stain in both nucleus and cytoplasm.

In all experiments observations upon the living cells were confirmed by a study of stained and fixed preparations. Diluted preparations and controls were, of course, fixed atthe same moment.

The results of these studies which show, in brief, that the amount of fat accumulated by cells in cultures varies directly with the fat content of the plasma medium, afford further evidence in favor of the view that these fatty accumulations are not degenerative in origin, but are the result of some disturbance in the metabolism of the cells.

82 (778)

The rate of absorption of water by the skin of the frog, in relation M. H. Fischer's theory of edema.

By J. F. McCLENDON.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

Fischer observed swelling of amputated frog's legs, in water. The question arises: is this a phenomenon of osmosis due to the osmotic pressure of cellular and intercellular fluids?

A frog's leg was tied tightly and amputated above the ligature. The second leg of the same frog was skinned and the skin filled with a 0.7 per cent. NaCl solution and tied at the same level as the first leg. The two legs were weighed, placed in water, and weighed at intervals to determine the water absorbed. The leg filled with NaCl solution absorbed water more rapidly than the other leg.

An amputated and ligatured leg was placed in 0.7 per cent. NaCl. Its weight remained constant.

The ratio of the skin areas of a whole frog except the head, to the hind legs below the knees, was found to be about 3.5. Two frogs of the same size were selected. The hind legs of one were tied just above the knees and amputated above the ligatures and placed in water. The other frog was put in a harness that kept the head out of water, and a canula with rubber bag attached was inserted into the cloaca. This experiment was repeated a large number of times. The water absorbed by the whole frog within 6 hours was always more than 3.5 times as much as that absorbed by the two hind legs. The water absorption for longer periods of time is being studied.

Conclusion.—The swelling of frog's legs, in which the circulation of the blood is stopped, may be accounted for by osmotic pressure.

83 (779)

The dynamics of a model of cell division.

By J. F. McCLENDON.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

A low beaker is half filled with distilled water and a funnel inserted so that the stem extends to the bottom. A saturated solution of NaCl is slowly poured into the funnel and forms a layer beneath the pure water. About 1 c.c. of a mixture of 2 parts chloroform and 3 parts rancid olive oil is sucked up into a pipette and injected into the beaker so that it forms a drop suspended between the NaCl solution and the pure water. Two pipettes with capillary openings are filled with 1/10 normal NaOH solution and inserted into the beaker. The NaOH solution is

allowed to flow onto opposite poles of the drop at the same time and rate. The drop quickly elongates toward the pipettes, *i. e.*, toward the poles, and constricts along the equator, and sometimes divides into two. The smaller the drop, the more certain the division, provided the operator has sufficient skill.

The alkali forms soap which reduces the surface tension on the polar areas, and the hydrostatic pressure within the drop causes these areas to bulge, whereas the relatively higher surface tension of the equatorial region causes it to constrict until a barrel-shaped figure is formed, which rapidly becomes hour-glass shaped. The equatorial surface film contracts and the polar surface film spreads, causing vortex movements. The enlargement of the polar fields spreads the soap over larger areas, and the area of unaltered surface tension is reduced to a narrow equatorial band. This band, being partially released by reduction of tension at its edges, acts as a sphincter and constricts until it cuts the oil drop into two. This constriction of the oil drop may be considered as a rough model of cell division.

T. B. Robertson in a recent paper[1] claims that exactly the opposite changes take place in cell division. He divided the oil dropp by lacing on it a linen thread 0.4 mm. in diameter, previously soaked in the alkali. If the drop is not more than 1/10 c.c. in volume the thread cuts it in two. This is due to gravitation of the thread, the alkali merely lessening the resistance to the cutting. I found that better results were obtained by adding a little alkali to the water instead of soaking the thread in it.

The various points in Robertson's argument cannot be considered here, and the reader is referred to his paper. The most striking fallacy is that in Fig. 2, p. 699, M_1 and M_2 are not the same distance below the curved line *alsmd*.

[1] *Arch. f. Entwicklungsmech.*, 1913, XXXV, p. 692.

84 (780)

Antitoxic action of sodium iodid on morphin.

By T. S. GITHENS and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

A Preliminary Communication.

The relation of iodids to morphin was studied by Reid Hunt. He found that feeding mice, rats and guinea-pigs with potassium iodid increases their susceptibility to morphin poisoning. This may be considered as the chronic influence of iodids. In our experiments we studied their acute effects. Our experiments were made, in the first place, on rabbits and sodium iodid in 5 per cent. solution was the salt employed. Morphin was administered intravenously. The iodid solution was given intravenously, and subcutaneously, ten to thirty minutes before the morphin injection. The quantity of sodium iodid administered to each rabbit was quite large; 15 cubic centimeters intravenously and 30 cubic centimeters subcutaneously. Such injections of sodium iodid alone seemed to cause no ill-effects in rabbits.

Morphin, if not rapidly fatal, causes narcosis, paresis, tremors, convulsions and finally death. The fatal dose of morphin for rabbits is somewhat variable, it is therefore difficult to study the influence which other substances may exert upon the toxic action of morphin. The most definite results we obtained have been with doses of 300 and 250 milligrams of morphin per kilo body-weight. Eighteen rabbits received morphin alone; 11 of these animals received the drug (300 milligrams in each case) through the ear vein, while in 7 animals the morphin (300 or 250 milligrams) was injected through the jugular vein. Sixteen rabbits received sodium iodid besides morphin. In ten of these animals the injection (300 milligrams) was given through the ear vein and in six through the jugular vein. The difference in the results was quite striking. Of the eighteen animals which received morphin alone, ten died immediately after the injection, five lived less than 2 hours, one lived four days and two survived. Of the sixteen rabbits which received sodium iodid besides morphin, only one

died immediately after the injection, three lived longer than three hours, *i. e.*, between 3 and 20 hours (died in night), one lived eighteen, one twenty-three, and one thirty-six hours; one lived two days, one nine and one 16 days, and six rabbits survived. The meaning is quite unmistakable; the injection of sodium iodid undoubtedly reduced the mortality or postponed death in a palpable manner. We may add that the favorable effect of the iodid seemed to be more manifest in white than in gray rabbits.

The experiments seem to demonstrate also that sodium iodid antagonizes essentially the tetanic effects of morphin, while the depression is perhaps even more manifest in the iodid animals. However, we shall not discuss these particulars for the present.

We experimented also with mice. For mice we can only say for the present that iodid seems to retard perceptibly the onset of convulsions and the fatal outcome of morphin poisoning.

85 (781)

On the nature of the semi-permeable membranes which surround the fibers of striated muscle.

By EDWARD B. MEIGS.

[*From the Wistar Institute of Anatomy and Biology.*]

The view that the fibers of striated muscle are surrounded by semi-permeable membranes has received a wide acceptance among physiologists, and there has been a good deal of speculation regarding the nature of these membranes. The hypothesis that they are composed of lipoids has received much attention. Artificial lipoid membranes, however, have been found to be either impermeable both to water and to dissolved substances or else nearly equally permeable to water and to dissolved substances. It is a general rule that artificial membranes composed of pure colloids are either impermeable to both water and dissolved salts; or else nearly equally permeable to water and salts, and impermeable only to colloids. The best known artificial membranes which are semi-permeable with regard to salts dissolved in water are composed of precipitates of insoluble salts such as copper ferrocyanide and calcium phosphate.

The animal body can present the conditions necessary for the precipitation of calcium phosphate, as, for instance, in the case of bone formation. The striated muscle fibers contain considerable amounts of dipotassium phosphate, and are surrounded by lymph which contains calcium chloride, so that it is far from inconceivable that thin layers of calcium phosphate might be precipitated at the surfaces of the muscle fibers.

I have examined some of the properties of celloidin membranes impregnated with calcium phosphate. Celloidin membranes free from precipitate are quite permeable both to water and to dissolved salts. Such membranes were filled with a dipotassium phosphate solution and immersed in a calcium chloride solution. Under these circumstances they become impregnated with calcium phosphate, and at the same time they become markedly semi-permeable with regard to salts dissolved in water. That is to say that if they separate salt solutions of different osmotic pressures, water passes rapidly from the less concentrated to the more concentrated solution.

Celloidin membranes impregnated with calcium phosphate were filled with a 1.3 per cent. solution of dipotassium phosphate and immersed in isotonic solutions of various substances: it was then determined whether or not water passed through the membrane from the outer solution to the inner one against a moderate hydrostatic pressure. Such experiments were tried with solutions of NaCl, KCl, $CaCl_2$, cane sugar, alanin (an amino acid), glycerine, urea, and ethyl alcohol. The results indicated that the membranes were highly impermeable to salt, sugar, and amino-acid, somewhat permeable to glycerine and urea, and highly permeable to ethyl alcohol. In all these respects they resemble the muscle membranes with the possible exception of the case of KCl. There is some reason to believe that the muscle membranes are more or less permeable to KCl.

The supposition that calcium phosphate plays a part in giving to the muscle membranes their semi-permeable properties would explain two great classes of facts—those facts, namely, which show that muscles rapidly lose their semi-permeable properties in acid solutions; and those which show that calcium may play an important part in maintaining the semi-permeable properties

of muscle. Precipitates of calcium phosphate are rapidly dissolved by acids, and would dissolve slowly in neutral calcium-free solutions. In view of these considerations I propose to devote some time to the study of the osmotic properties of calcium phosphate precipitates, as well as to those of precipitates of calcium carbonate and of magnesium phosphate and magnesium carbonate.

86 (782)

The mechanical factors of excessive artificial respiration and a consideration of their relation to the acapnial theory of shock.

By **H. H. JANEWAY** and **E. M. EWING.**

[*From the Department of Physiology, Univeristy and Bellevue Hospital Medical College.*]

It has been claimed that the most important factor in the causation of shock is diminution of CO_2 within the blood, and that this diminution is a regular consequence of all influences resulting in shock. That CO_2 possesses important physiological functions cannot be denied. An investigation therefore of the true significance of a diminution of its normal amount within the blood is important and bears a special relation to various methods of artificial respiration utilized in thoracic surgery. The present experiments were undertaken for the purpose of investigating the effects of acapnia and the relation of some factors concerned in its production to shock. In all of them dogs were used. The first series was performed for the purpose of studying the effect of variations in intrapulmonic air pressure upon the blood pressure. The thorax was opened laterally, a T-tube connected with a water manometer was tied in a small bronchus, and the heart enclosed in a Henderson cardiometer in series with a recording tambour. The blood pressure was recorded from the carotid artery. The thorax was closed and the animal was subjected to intratracheal insufflation from an apparatus provided with an exhaust valve which reduced the pressure to approximately zero about four (4) to twelve (12) times per minute. The blood pressure averaged 150 mm. Hg. when the intrapulmonic air pressure was not allowed to exceed 6 mm. Hg.

In one experiment, with an increase of intrabronchial pressure from 8 mm. to 30 mm. Hg, blood pressure fell from 122 mm. to 55 mm. Hg and the volumetric tracing indicated that the output from the heart had diminished about 44 per cent. These variations in blood pressure were completed within a few seconds after the change in intrabronchial pressure, and could be duplicated at will.

A rise of intrabronchial pressure above 8 to 10 mm. Hg always caused a fall in blood pressure, and it was concluded that the variation in pressure was the result of a diminished venous return to the heart, resulting from compression of both the pulmonary and systemic veins in the thorax.

In view of the marked changes in heart output and blood pressure resulting from small variations in intrapulmonic pressure, it is evident that, in any experiments attempting to estimate the part played in the production of shock by a diminution of CO_2 which is caused by hyperartificial respiration, the effect of the increase of intrapulmonic pressure upon the return flow of blood to the heart must be considered.

In the second series of animals, Henderson's experiments were duplicated, the dogs being artificially respired by means of a force and suction pump, working about 70 times per minute. The animals were given morphine, and ether was administered only when necessary. In these experiments, blood pressure fell about 40 per cent. within one minute after artificial respiration was begun, and then decreased more slowly throughout the experiment to between 40 and 50 mm. Hg. At the end of the experiment when the artificial respiration was stopped, blood pressure increased 60 to 90 per cent. within a few seconds. In all experiments the blood gas analysis showed the CO_2 content at the end to be only 40 to 50 per cent. of the original volume. These animals, at the end of 2 to 3 hours of artificial respiration, were all in a condition of deep shock. This degree of shock was indicated by a rapid pulse, a marked degree of coma, and insensibility to a sensory stimulation. Three of the animals so treated lived three days (dying of secondary effects of the experiment), and one lived 24 hours. None of them died from the immediate effects of the experiment. During these experiments when the artificial respiration was

temporarily stopped or permanently stopped at the end of the experiment the period of apnea lasted only one or two minutes, so that no death resulted directly from acapnia. The absence of a prolonged period of apnea is explained by the fact that the effect of ether was not added to that of morphine.

With a third series of animals, the experiments just described were duplicated with the exception that the CO_2 content of the blood was maintained at its normal level, or slightly above it. The conservation of CO_2 was accomplished by inserting a large rubber bag, to act as a reservoir, between the suction pump and the force pump, thus creating an almost perfectly closed circuit; the dog thus rebreathed expired air. To replace a small amount of air lost from the animal's trachea, CO_2 was fed from a tank into the rubber bag where the latter was diluted with air drawn in from the trachea in smaller quantities than that amount lost through this route. In these experiments the animals went into the same degree of shock in 2 or 3 hours as those of Series II in which the CO_2 content of the blood was diminished to 40 per cent. of the original volume. One animal died on the table just before the experiment closed, the others lived one to three days. Blood pressure changes in the two series were similar, but a characteristic of the experiment when the CO_2 was kept high, was the slow, strong heart beat, in place of the rapid, weak pulse observed when the CO_2 was diminished. Conclusions drawn from Series II and III can only be that the reduction in the CO_2 content of the blood is not the important factor in the production of shock by hyper-respiration, but that in the shock so produced, the essential factor is an interference with the venous return to the heart.

In the fourth series of experiments the effects of aeration and handling of the intestines were studied. A celluloid window was placed in the abdominal wall, and a stream of warm, moistened air was passed over the intestines for a period of three hours. During this procedure the animals breathed normally, the blood pressure was 163 mm. Hg, CO_2 content was normal, and there was no evidence of shock. The celluloid was then removed, the intestines spread out, and the aeration continued. After 45 minutes the CO_2 determination showed 38.8 volume per cent., and blood pressure was 153 mm. Hg. The intestines were then

handled, and in ten minutes blood pressure had fallen to 98 mm., and in 20 minutes to 56 mm. Hg. In 40 minutes there was still 31.6 vol. per cent. CO_2 in the arterial blood.

In another experiment the intestines were exposed and aerated (not handled). The CO_2 content of the blood was maintained by connecting a long tube to the trachea. After one hour and a half, blood pressure had changed but one mm. Hg and the animal was in good condition. The intestines were then handled, and in ten minutes the blood pressure fell from 122 to 60 mm. Hg. The CO_2 content was 45.1 per cent. In 25 minutes the blood pressure was 46 mm. Hg, the CO_2 content normal, and the dog in shock to such a degree that the sciatic nerve could be dissected out without the administration of ether, the animal making no movement whatever. In these experiments on the abdominal cavity the primary factor concerned is unquestionably the manipulation of the intestines, and not any diminution of CO_2 caused thereby. It will be remembered that in the similar experiments of aeration of the intestines, reported by Henderson, the intestines were "handled gently." We have been unable to find that he notes any mention of aerating the abdominal cavity beneath a celluloid membrane with air as a control experiment.

His control experiment, in which he did not secure shock, was merely aerating the abdominal cavity beneath a celluloid window placed in the abdominal wall with a stream of air plus CO_2.

The present experiment shows that aeration of the intestines without the addition of CO_2 does not produce shock.

87 (783)

A note on the transmission of spirochætes.

By JOHN L. TODD.

Leishman and other authors have shown that the anal and coxal excretions voided by ticks (*Ornithodoros moubata*), capable of transmitting spirochætes (*Spirochæta duttoni*), are infective and that susceptible animals inoculated with these fluids develop a spirochætal infection. It has been asserted that animals upon which infected ticks have fed will not become infected, unless

these fluids are voided while the ticks feed, and it has been suggested that the infection is transmitted by the flowing of the fluids into the wounds made by the mouth-parts of the ticks in feeding. Spirochætes had not been seen in these fluids and it was suggested that they existed there in a coccoid form.

On several occasions, coxal and anal fluids, excreted by infected ticks, coming from Uganda and British Central Africa, have been examined. In every instance the fluid was taken while the ticks fed upon an uninfected animal. The fluid collected was free from blood and, in two instances, coxal fluid was collected apparently free from anal excretion. On six occasions, after the fluid had been centrifugalized, spirochætes were found in it; their morphology is not distinct from that of *Spirochæta duttoni*. Spirochætes were found in the fluid that apparently contained no anal excretion.

88 (784)

Experimentally fused larvæ.

By A. J. GOLDFARB.

[*From the Department of Natural History, The College of the City of New York.*]

When the eggs of *Toxopneustes variegatus* were subjected to a 5/8 molecular NaCl, after the removal of the fertilization membrane, considerable numbers were subsequently fused together. I have counted as many as forty percentum, in the optimum solutions, of agglutinated and fused pairs, triplets, etc. Few of these reached the pluteus stage of development due to the early death of all fusions of more than three eggs, and to the large mortality of even the double embryos.

The plutei contain at least three characteristic tissues, namely, body wall, archenteron, skeleton. The first two of these behaved essentially as described by Driesch in various European species, and by the writer in the American species *Arbacia punctulata; i. e.*, the body walls or the archentera of plutei derived from separate eggs were united either incompletely or so completely as to give little or no evidence of the original dual character of the larvæ.

It was supposed that the skeletal structures united in the same manner as the archentera. The evidence furnished by *Toxopneustes* however clearly shows that in this species at least no fusion of the skeletal parts occurred. Instead some very interesting changes took place which may be stated briefly as follows: One of the pair of fused larvæ developed normally in every detail, the other developed in nearly every instance, incompletely. An almost perfect series of fused larvæ were obtained in which the incomplete pluteus lacked more and more of the characteristic parts that constitute the perfect larval skeleton; and the order of their disappearance was in the reverse order of their appearance in ontogeny.

The union of the two larvæ involved the approximation of their branched and complex skeletons, whose parts frequently overlapped but never fused.

89 (785)

Metabolism studies in a case of myotonia atrophica.

By JACOB ROSENBLOOM and BENSON A. COHOE.

[*From the St. Francis Hospital, and the Laboratory of Biochemistry of the University of Pittsburgh, Pittsburgh, Pa.*]

In a thirteen day metabolism study on an individual suffering from myotonia atrophica, we have studied the nitrogen metabolism, and urinary nitrogen partition, the sulphur metabolism and urinary sulphur partition, and the calcium, magnesium, phosphorous, chlorine and fat metabolism. The creatinine excretion was normal. The only striking metabolic anomaly noted in this study was the marked loss of calcium.

90 (786)

Sugar from lactic acid in human diabetes.

By NELLIS B. FOSTER, M.D.

[*From the Medical Service of The New York Hospital.*]

The evidence presented by experimental diabetes seems to indicate that in the transformation of amino-acids into glucose lactic acid is an intermediary step. It has also been suggested

that in the combustion of glucose lactic acid may arise from the cleavage of the sugar molecule. On account of this significant place held by lactic acid it appears of interest to investigate the relation it may hold to sugar production in human diabetes. Only cases of diabetes of considerable severity are suitable for the experiment, since in the milder degrees of the human disorder relatively large amounts of carbohydrate may be burned or stored and the sugar output is apt to fluctuate. In a patient it was ascertained that with a uniform diet for three days a fairly constant glucose excretion could be depended upon and lactic acid as the sodium salt was given on the second day of the experimental period with the result that there was a rise in sugar excretion shown by the following abstract from the protocol.

	Amount urine.	Sugar, grams.	Total N.	NH_3N.	Acetone and diacetic.	B-oxy butyric.
Vegetable diet....	1,810	2.89	3.62	1.37	.78	0.79
Oatmeal diet.....	1,450	2.80	4.80	1.22	.59	1.41
Meat diet.....	2,300	6.56	11.46	1.42	.76	1.77
15 gm. Na lactate.	2,010	19.90	14.06	1.56	.82	1.49
	2,050	2.91	14.92	0.71	.86	1.97

91 (787)

On some vaccinia blood pressor substances in rabbits.

By J. P. ATKINSON and CHAS. B. FITZPATRICK, M.D.

[*From the Chemical and Research Laboratories, Department of Health, City of New York.*]

These notes constitute a continuation of our observations on some blood pressor substances in experimental immunity, reported at the last meeting of this society, and apparently tend to confirm the same. We have endeavored to ascertain when the pressor and depressor substances appeared in the blood serum of living rabbits after they had been successfully infected with vaccinia virus. The height of the production of the vesicles from which the virus was collected was reached on the fifth day. They then rapidly healed and the rabbits appeared outwardly to be in good health.

Observations were also made to ascertain the effect of aging on the presence of the depressor substance.

TABLE I.

Showing when the pressor and depressor substances appear.

Date of experiment.	Time after inoculation.	Drawn from clot.	Amount of injection.	Reaction.
March 8, 1913.	48 hours. No vesicles (bled 3–7–13).	Within 12 hours of bleeding.	7 c.c.	A good depression.
March 8, 1913.	48 hours. No vesicles (bled 3–7–13).	Within 12 hours of bleeding.	8 c.c.	A very marked depression.
March 28, 1913.	5 days[1] (bled 3–10–13 quite red).	Within 48 hours of bleeding.	$2\frac{1}{2}$ c.c.	Decided rise in pressure, followed by clotting.
March 28, 1913.	5 days[1] (bled 3–10–13 quite red).	Within 48 hours of bleeding.	5 c.c.	A good rise in pressure.
March 28, 1913.	5 days[1] (bled 3–10–13 quite red).	Within 48 hours of bleeding.	5 c.c.	A good rise in pressure.
March 28, 1913.	8 days (bled 3–22–13).	Within 4 days of bleeding.	8 c.c.	Fine marked double depression.
March 28, 1913.	10 days (bled 3–15–13), quite red.	Within 4 days of bleeding.	$2\frac{1}{2}$ c.c.	Slight rise followed by a slight fall.
March 28, 1913.	12 days (bled 3–17–13).	Within 4 days of bleeding.	4 c.c.	Slight rise followed by a slight fall.
March 28, 1913	17 days (bled 3–22–13).	Within 4 days of bleeding.	5 c.c.	Marked depression.
March 28, 1913.	30 days (bled 3–22–13).	Within 4 days of bleeding.	5 c.c.	Slight rise followed by a slight fall in pressure.

[1] This 5 days result may also have changed to pressor, during the 16 days the serum was kept in the ice-box.

TABLE II.

Showing the effect of aging on the presence of the pressor substances.

Date of experiment.	Time after inoculation.	Drawn from clot.	Amount of injection.	Reaction.
March 8, 1913.	48 hours. No vesicles. Bled 3–7–13.	Within 12 hours of bleeding.	7 c.c.	Good depression.
March 8, 1913.	48 hours. No vesicles. Bled 3–7–13.	Within 12 hours of bleeding.	8 c.c.	Very marked depression.
March 28, 1913.	48 hours. No vesicles. Bled 3–7–13.	Within 12 hours of bleeding.	1 c.c.	No reaction.
March 28, 1913.	48 hours. No vesicles. Bled 3–7–13.	Within 12 hours of bleeding.	6 c.c.	Decided rise.[1]
March 28, 1913.	8 days. Bled 3–22–13.	Within 4 days of bleeding.	8 c.c.	Fine, marked double depression.
April 4, 1913.	8 days. Bled 3–22–13.	Within 4 days of bleeding.	8 c.c.	Slight rise followed by slight fall in pressure.[2]
March 28, 1913.	17 days. Bled 3–22–13.	Within 4 days of bleeding.	5 c.c.	Marked depression.
April 4, 1913.	17 days. Bled 3–22–13.	Within 4 days of bleeding.	8 c.c.	Quite a decided rise, followed by an equal fall in pressure.[3]
March 28, 1913.	30 days. Bled 3–22–13.	Within 4 days of bleeding.	5 c.c.	Slight rise followed by slight fall in pressure.
April 4, 1913.	30 days. Bled 3–22–13.	Within 4 days of bleeding.	8 c.c.	Marked rise in pressure.

[1] *I. e.*, after standing in the ice-box 20 days the depressor disappeared and pressor appeared.
[2] *I. e.*, after standing in the ice-box 13 days the depressor was partially eliminated.
[3] *I. e.*, same result as the preceding.

Summary.—1. Depressor and pressor substances arise after vaccinia infection in the blood-serum of rabbits. 2. Aging tends to eliminate the depressor substance and a pressor substance then comes in evidence.

Note.—A serum obtained from a rabbit after streptococcus infection, which had 12 months previously given a profound depressor reaction was also tested (3–18–13) and was found to give no reaction. 8 c.c. of a saline extraction of the adrenals of a 30 day vaccinia rabbit, gave (3–28–13) no reaction. This extraction was made in 20 c.c. of physiological saline solution and was kept 6 days in the ice-box. 7 c.c. of a saline extraction of the adrenals of a two day vaccinia rabbit gave (3–8–13) a fine rise followed by a marked fall. This extraction was made in 20 c.c. and was kept 24 hrs. in the ice-box, *i. e.*, since immediately after removal.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

Third meeting.

San Francisco, California, April 2, 1913.

92 (788)

Preliminary communication on the part played by cholesterol in determining the incidence of carcinoma.

By **T. BRAILSFORD ROBERTSON** and **THEODORE C. BURNETT.**

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

We have elsewhere shown[1] that cholesterol, when injected directly into rat carcinomas, causes a marked acceleration both of the primary and of the metastatic growth of the tumors.

This led us to form the opinion that cholesterol is probably a factor of importance in determining the incidence of carcinoma.

It has been shown by Dorée and Gardner, Ellis and Gardner, and others[2] that cholesterol is not synthesized by animals, the

[1] T. Brailsford Robertson and Theodore C. Burnett, PROC. SOC. EXPER. BIOL. AND MED., 10 (1912), p. 59; *Journal of Exper. Med.*, 17 (1913), p. 344.

[2] C. Dorée and J. A. Gardner, *Proc. Roy. Soc. London*, 80 *B* (1908), p. 227; 81 *B* (1909), G. W. Ellis and J. A. Gardner, *ibid.*, 81 *B* (1909), p. 129.

cholesterol in animal tissues being derived from their diet. This fact suggested the possibility that the incidence of carcinoma in inoculated animals might be diminished by feeding them for a considerable period prior to the inoculation upon a diet poor in cholesterol.

Accordingly twenty-two white rats, about two months old, were divided without exercising any selection into two lots. One lot of 15 were fed upon a diet composed exclusively of milk; the remainder were fed upon a mixed diet of oats and meat.

The content of cholesterol in milk, while by no means negligible, is extremely small in comparision with the content of cholesterol in other foodstuffs. Thus Tolmatscheff finds that human milk contains from 0.025 per cent. to 0.039 per cent. of cholesterol,[1] while Bömer and Kirsten find that the fats in cow's milk contain 0.5 per cent. cholesterol, corresponding to a content of less than 0.02 per cent. in the whole milk.[2] Meat, on the other hand, contains from 0.07 to 0.08 per cent. of cholesterol,[3] while the content of phytosterols in seeds is considerable.[4]

Both lots of animals thrived well, the milk-fed animals presenting an especially well-nourished appearance.

At the end of two months both lots of rats were inoculated in the axillary region with portions of a Flexner-Jobling carcinoma. The diet of each lot of rats was maintained unaltered. At the end of twenty days the proportion of successful inoculations in each batch of animals was determined, with the following results:

Milk diet: 10 out of 15 = 67 per cent.
Mixed diet: 7 out of 7 = 100 per cent.

Another batch of half-grown animals obtained at the same time from the same dealer, which were fed upon a mixed diet and inoculated with Flexner-Jobling carcinoma yielded the following results:

Mixed diet: 55 out of 64 = 86 per cent.

While the milk-fed animals yielded the lowest percentage of successful inoculations it is evident that the difference between

[1] Tolmatscheff, *Hoppe-Seyler's Med.-Chem. Unters.*, 2 (1867), p. 272.

[2] R. W. Raudnitz, *Ergeb. d. Physiol.*, 2, I (1903), p. 264.

[3] C. Dormeyer, *Arch. f. d. ges. Physiol.*, 65 (1896), p. 99; G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 81 *B* (1909), p. 507.

[4] F. Czapek, "Biochemie der Pflanzen," Jena, 1905, Vol. I.

the milk-fed and the normal animals in this respect was not so marked as one would be inclined to anticipate if cholesterol were really a prime factor in determining the incidence of carcinoma, and if feeding for two months upon a diet unusually low in cholesterol really brought about any appreciable diminution in the cholesterol-content of the tissues.

That cholesterol is an important factor in determining the incidence of carcinoma can hardly be doubted in view of our previous results, cited above, and of the fact, recently discovered by Wacker, that the cholesterol content of the fatty deposits in the subcutaneous tissues and mesenteries of persons who have carcinoma is no less than 66 per cent. greater than the cholesterol content of the fatty deposits in normal persons.[1] An increase in the cholesterol content of reserve-fats was also observed by Wacker in aged persons and in persons afflicted with tuberculosis or diabetes.

Our failure to observe a more striking difference between the incidence of carcinoma in milk-fed and in normal rats we are inclined to attribute to the fact which has been demonstrated by Ellis and Gardner[2] that cholesterol is strictly conserved in the animal economy, since the cholesterol which is excreted in the bile is reabsorbed in the intestine and does not appear in the feces, while, on the other hand, cholesterol contained in the food is in large part absorbed. Hence removing cholesterol from the diet of an animal does not lead to an appreciable removal of cholesterol from the tissues. More conclusive results might be anticipated if animals were fed from the time of birth upon a cholesterol-free diet, since the tissues of newly-born animals contain relatively little cholesterol.[3] Experiments in this direction are being undertaken.

In conclusion we wish to point out that since the cholesterol in the diet is partly absorbed, while the cholesterol content of the body is strictly conserved, it follows that animals must tend to accumulate cholesterol. We believe that this explains the excessive cholesterol content of the reserve fats in aged persons which

[1] L. Wacker, *Zeit. f. physiol. Chem* , 80 (1912), p. 383.
[2] G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 81 *B* (1909), p. 505.
[3] G. W.Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 84 *B* (1912), p. 461.

has been observed by Wacker, and that this in its turn is the reason for the well-known increase in the incidence of carcinoma with advancing age.

93 (789)

The influence of digitonin upon the growth of carcinoma.

By T. BRAILSFORD ROBERTSON and THEODORE C. BURNETT.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

It has been shown by Windaus[1] that digitonin combines with cholesterol to form a very insoluble and pharmacologically inactive compound. In view of the marked influence of cholesterol in accelerating the growth of carcinoma we have thought it of importance to ascertain the influence of digitonin upon the growth of Flexner-Jobling carcinoma in rats.

The digitonin employed was Merck's, stated to have no physiological action upon the heart. By heating the preparation to boiling in m/6 NaCl solution a soapy-looking fine suspension is formed which settles out in the course of several hours. We injected the digitonin, suspended either in m/6 NaCl, or in m/6 NaCl containing 1 per cent. of lecithin, directly into the tumors.

One hundred and sixty-six white rats were inoculated with Flexner-Jobling carcinoma in the axillary region. The number of successful inoculations, determined after 20 days, was 64, or 39 per cent.

On the 20th day after inoculation these animals were sorted, without selection, into three batches, of which one (consisting of 12 animals) served as controls, another (12 animals) received injections of digitonin, and the third (40 animals) received injections of digitonin together with lecithin.

We began by administering 1 c.c. of a 1 per cent. suspension of digitonin, suspended in m/6 NaCl and in m/6 NaCl + 1 per cent. lecithin respectively. The animals which received digitonin without lecithin evinced symptoms of severe local irritation, and one

[1] A. Windaus, *Ber. d. d. Chem. Ges.*, 42, I (1909), p. 238; *Zeit. f. physiol. Chem.*, 65 (1910), p. 110; M. T. Fraser and J. A. Gardner, *Proc. Roy. Soc. London*, 82 *B* (1910), p. 559.

of the animals which received digitonin alone and two of those which received digitonin and lecithin died within a few hours after the treatment. Post-mortems showed that the heart had in each case stopped in extreme systole, the auricles being engorged. In subsequent treatments the dose of digitonin was reduced to 1 c.c. of a 0.5 per cent. suspension, and in the case of the animals which received lecithin, the concentration of the lecithin emulsion was increased to 2 per cent. In the course of these treatments two more of the animals which received digitonin and lecithin died, in each case post-mortem examination showed that the heart had stopped in extreme systole.

Treatments were given on the 20th, 22d, 25th, 27th, and 29th days after the inoculation.

It was found that twenty-four hours after the first treatment the tumors were soft and pulpy and also presented a discolored appearance. The tumors rapidly hardened again, however, and those which were treated with digitonin alone grew much more rapidly than the controls or the tumors which were treated with digitonin and lecithin together. This is shown by the following figures, the diameter of the tumors at the beginning of treatment being taken as 100 in each case.

	Diameter of the tumors.	
	20 days after inoculation.	25 days after inoculation.
Controls	100	114
Digitonin	100	146
Digitonin + lecithin	100	113

It is evident that lecithin antagonizes the action of digitonin in accelerating the growth of carcinoma. This we have confirmed by further experiments which will be reported in a later communication.

Thirty per cent. of the controls, thirty per cent. of the animals which had received digitonin alone, and twenty-five per cent. of the animals which received digitonin together with lecithin had undergone "spontaneous cure" 32 days after inoculation.

In order to ascertain whether or not the above effects were due to digitonin itself or to some impurity contained in Merck's preparation, we prepared pure digitonin by dissolving Merck's digitonin in alcohol, pouring this solution into a large volume of

water, collecting the precipitate, washing it in water and drying over H_2SO_4 at 36° C. The substance which was thus obtained was pure white. It is soluble in alcohol and forms unstable suspensions in water. One c.c. of a 1 per cent. suspension injected subcutaneously in rats produced no symptoms whatever, either local or general. A solution in alcohol precipitated cholesterol from alcoholic solution, and a suspension in water coagulated a suspension of cholesterol in N/100 sodium oleate.

Fourteen rats with carcinoma, which had been inoculated 20 days previously, were divided without selection into two lots, the one lot of 5 served as controls, the other of 9 received 1 c.c. of a 1 per cent. suspension of the above preparation of digitonin in m/6 NaCl, injected directly into the tumors on the 20th, 21st, 22d, 23d, 25th and 26th days after inoculation. As in the previous experiment, the earlier injections caused softening and discoloration of the tumors. The tumors soon hardened, however, and thereafter grew much more rapidly than the tumors in the controls, as the following figures reveal:

	20 days after inoculation	25 days after inoculation.	29 days after inoculation.
Controls	100	110	126
Digitonin	100	147	168

We interpret these results in the following way: When digitonin is injected into the tumor it first of all renders inactive the cholesterol then within the tumor and this results in softening and incipient degeneration of the tumor. The precipitated cholesterol is, however, replaced by cholesterol from the surrounding tissues and body-fluids, and the cholesterol bound by the digitonin is retained within the tumor and gradually liberated from its combination with the digitonin, with the result that cholesterol is concentrated within the tumor, and the growth of the tumor is thereby accelerated. The fact that lecithin prevents the acceleration of the growth of the tumor by digitonin is in harmony with the above interpretation and with our previous results showing that lecithin and cholesterol have opposite actions upon the growth of carcinoma.[1]

[1] T. Brailsford Robertson and Theodore C. Burnett, Proc. Soc. Exper. Biol. and Med., 10 (1912), p. 59; *Journal of Exper. Med.*, 17 (1913), p. 344.

94 (790)

Note on the relation of alexin to thrombin.

By THEO. C. BURNETT (by invitation).

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

The publishing of negative results is, as a rule, a questionable proceeding. Occasionally however, a negative result may have a positive bearing upon a subject, of more or less value, in which case the objection does not hold. It is for this reason I present the following note.

In reviewing the subject of coagulation some time ago, I was struck by a certain similarity between thrombin and alexin. Both are inactivated by a temperature of 56 degrees; both are derived from leucocytes;[1] thrombin consists of thrombokinase and calcium, while alexin consists of two parts, as is well known. It occurred to me that there might be a closer relation between the two; that they might in fact, be identical. It has probably occurred to many another, but in a hasty glance over the literature, I can find no mention of it. At first sight the idea seems absurd, for alexin is contained in serum that has been collected over a clot, and hence contains no thrombin. The alexic potency of a serum, however, increases by standing for some hours in contact with the clot, and it is conceivable that during that time thrombin is excreted by the leucocytes, but is not apparent because of the absence of fibrinogen wherewith to combine. Having occasion to prepare some thrombin for another purpose, I determined to test the matter.

The immune serum used was rabbit serum immunized against ox corpuscles. Tested with guinea-pig serum it had a potency of 1–800. (Ox blood was used on account of the ease with which material could be obtained at any time.) Most of the experiments were carried out with a 1–100 dilution, although similar results were obtained with higher dilutions.

The thrombin was prepared by Howell's method[2] and gave a

[1] It is generally conceded now that thrombin is derived from the platelets.

[2] Howell, W. H., *Am. Jour. Physiol.*, Vol. 26, 1910, p. 453.

coagulum with fibrinogen in a few minutes. As thrombin is made with 8 per cent. NaCl solution, it was diluted to approximate isotonicity, m/6. In order to still further control any possible osmotic effects from hypotonicity, solutions of m/5 and m/4 concentrations were also used to dilute the serum.

The system consisted of 1 c.c. of 1–100 immune serum, plus 0.1 c.c. washed ox corpuscles (5 per cent. emulsion), plus thrombin in amounts varying from 0.05 c.c. to 0.3 c.c. As a control one tube was always prepared with normal serum (alexin) instead of thrombin. The tubes were kept at a temperature of 36–37° C.

The results can be stated in a few words. The control tubes showed complete hemolysis in from fifteen to thirty minutes. In no instance was there a trace of hemolysis even after several hours, in the tubes containing thrombin. The corpuscles settled out of the solution and left a clear supernatant fluid, not even tinged with hemoglobin. Having obtained this negative result with thrombin, I then tried solutions of fibrinogen and of serum-globulin, in order to make the matter complete. Exactly the same results were obtained, and I think we may conclude that whatever alexin may be, it is certainly *not* identical with thrombin or the globulins.

95 (791)

On the nature of the union of alexin with specific precipitates.

By HANS ZINSSER, M.D.

[*Division of Bacteriology, Medical Department, Stanford University.*]

In continuing studies on the nature of alexin fixation by mixtures of unformed proteins and their antisera, it occurred to the writer to examine whether the alexin fixation which is exerted by specific precipitates was subject to the same conditions that prevail in the case of sensitized cell complexes in their relations to the alexin fractions as first obtained by Ferrata. It is well-known, of course, that by dialysis, by dilution with weak acid in distilled water and by a number of other methods of globulin precipitation, the alexin or complement can be divided into two functional parts, one which comes down with the globulins, the

so-called "midpiece" and the other which remains in the albumin fraction, the so-called "endpiece." Neither of these can produce hemolysis of sensitized cells alone. Together they functionate. The globulin fraction can be bound to sensitized cells, forming the so-called "persensitized cells" which are now hemolyzable by the endpiece alone. The albumen fraction does not become attached or fixed to the sensitized cells except in the presence of the globulin fraction. (The terms midpiece and endpiece are used for the sake of clearness since they are terms which have become established in the German literature. Owing to studies which are being made by Mr. Maltaner in this laboratory we feel that a definite nomenclature which assumes an intermediate function of the globulin, fraction, is premature.)

In experiments in which alexin fractions, produced by both the method of Ferrata and by that of Sachs and Altmann, were exposed to union with precipitates, formed in mixtures of beef serum and its antiserum, we have found that the conditions which prevail are entirely analogous to those which govern the attachment of the alexin fractions to the sensitized cells. A specific precipitate may fix the globulin fraction (midpiece) alone. It may fix the albumin fraction (endpiece) in the presence of the globulin fraction. It does not however, fix the albumin fraction (endpiece) by itself. The experiments were in every case controlled by titrations of the alexin fractions and the whole alexin in tubes set up parallel with the main experiment.

The writer believes that these results have considerable theoretical importance in bearing out his previously expressed view that the "precipitin" may be regarded as a protein sensitizer, the fact of visible precipitation being a merely secondary occurrence due to the union of two colloids under conditions of quantitative relations and environment which favor precipitation.

96 (792)

Action of gentian violet on Mucosus capsulatus group.

By J. G. FITZGERALD and GERTRUDE MACKINTOSH.

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

It has recently been shown by Churchman[1] in two interesting communications bearing on the subject that the aniline dye, gentian violet, has a selective bactericidal action on certain bacteria. The action of the dye has been spoken of as bacteriostatic, indicating that the growth of some species of bacteria is inhibited. In addition to this inhibiting influence, Churchman believes the substance has also a very definite bactericidal action. It was shown in the publications referred to that the action of gentian violet as a bacteriostatic or bactericidal agent presented in a general way a parallelism with the Gram stain. The majority of gram positive bacteria are inhibited by gentian violet, while the majority of gram negative bacteria are not. The action of gentian violet can be observed on the divided plates or by staining with gentian violet and determining subsequently whether the microörganisms so treated will grow on culture media. On divided plates, one half of an ordinary petri dish is covered with plain nutrient agar, the other half with nutrient agar to which has been added an aqueous solution of gentian violet; such a plate when streaked with gram positive bacteria will show a growth only on the side of the plate where there is no gentian violet, while gram negative bacteria usually grow equally well on the plain agar and on gentian violet agar.

Since it seemed possible that the differentiation of closely related species might be accomplished by the use of divided gentian violet plates, the method has been used in a further study of the *Mucosus capsulatus* group. In all, thirty-six strains of bacteria have been investigated; they include *B. ozænæ*, *B. lactis aerogenes*, *B. enteriditis*, *B. pneumoniæ* Friedlander, *B. rhinoscleromatis*, *B. capsulatus*, and *B. Mucosus capsulatus*. All of the

[1] Churchman, *Journal Experimental Medicine*, Vol. XVI, No. 2, August, 1912, pp. 221–247, and Vol. XVI, No. 6, December, 1912, pp. 822–830.

thirty-six were found to be gram negative. This is in harmony with most of the observations heretofore recorded, although a contrary statement is made with reference to *B. rhinoscleromatis* by McFarland[1] and by Page.[2] When studied by means of the gentian violet agar plates, twenty-nine of the thirty-six cultures were found to grow equally well on plain agar and on gentian violet agar. Seven others, however, behaved differently. No growth could be obtained on the gentian violet agar side of the divided plates. Churchman has mentioned the fact that certain bacteria when stained will not grow on one culture medium but might grow on a more selective medium. The seven strains that refused to grow on ordinary gentian violet agar were then planted on serum agar violet, and three of them grew on serum agar violet, although they had refused to grow on ordinary violet agar. The other four did not grow even on the more favorable medium and behaved in the same way as most of the gram positive bacteria. Thinking the substitution of a selective culture medium for ordinary medium in the case of certain of the gram positive bacteria might result in a change in the action of gentian violet on their growth, *B. diphtheriæ* and streptococcus were planted on serum agar violet plates, and on agar violet plates. Both plates failed to show a growth on the violet side.

Of the three microörganisms that grew on serum agar violet, two have remained constant, growing well on serum agar violet but not at all on ordinary agar violet; the third one, however, has shown a very interesting adaptation. The culture is one of *B. pneumoniæ* Friedlander from the Kral collection. This bacillus when grown on a plate containing serum violet and agar violet can be induced to grow on agar violet once it has started to grow on serum violet; if, however, the growth is started from the opposite side of the plate on plain agar, it cannot be made to grow on violet agar. The experiment was made by putting in one plate culture, serum agar, serum agar violet, agar violet, and plain agar, the different media merging into one another in a petri dish. The behavior of the microörganism is thus shown to be inconstant toward gentian violet, depending on the culture media on which

[1] McFarland, "Pathogenic Bacteria and rotozoa," 7th edition, 1912, p. 785.
[2] Page, *Journal Medical Research*, Vol. XXVI, No. 3, July, 1912, pp. 489–499.

it is grown, that is, a microörganism incapable of growing in the presence of gentian violet readily does so on a more suitable medium, and it can adapt itself to a less suitable medium if started on a selective medium. Gentian violet has not been found to be of any value in the differentiation of the *Mucosus capsulatus* group. Further, the microörganisms of this group which refuse to grow on agar violet are representatives of what we at present regard as three distinct species.

It would seem from the result of the experiment here recorded that while the action of gentian violet on bacteria is usually constant, it is sometimes susceptible of modification and may not be as fundamental a characteristic as originally supposed.

97 (793)

On the influence of light on the electric potential of bacterial and other suspensions.

By S. W. YOUNG (by invitation).

[*Laboratory of Physical Chemistry, Stanford University, Cal.*]

Experiments in this laboratory have shown that light and other forms of radiant energy exert a marked influence on the rate of wandering in the electric current. Thus the rate of wandering of arsenic sulphide suspensions is about twice as great in the dark as under strong illumination in the sun, or in the carbon arc or in the Nernst lamp. On the other hand, the rate of wandering of mastic emulsions is increased under strong illumination, and that to the extent of about forty per cent. of the value in the dark. Ferric hydroxide is retarded in the light to the extent of about six per cent., while chlorophyll suspensions are accelerated in the light to the extent of about forty per cent. The effect of the radiant energy is not in general instantaneous, but requires a few minutes exposure to reach its greatest value. In general also the effect is reversible, that is colloids whose rates of wandering have been influenced by radiant energy, return to their original values if kept in the dark for some minutes.

These phenomena are very interesting in connection with photochemical reactions in general. Arsenic sulphide suspensions

may be kept almost indefinitely in the dark. On exposure to light they oxidize rapidly. Knowing now that light also reduces the negative charge on the colloidal particles of arsenic sulphide, one can arrive at a comparatively simple electrical theory of photo-oxidation. If one assumes that oxidation takes place when two hydroxyl ions can give up their charges, forming water and nascent oxygen available for oxidation, and that subsequently the negative charges thus set free may react with free hydrogen ions and free oxygen to form new hydroxyl ions which may again discharge and oxidize, it will be readily seen that the rate of oxidation will primarily depend on the value of the negative charge which the oxidized particle itself carries. If this is large the hydroxyl ions will be repelled, and oxidation will be retarded. If any force intervenes to reduce the negative potential of the colloid particle, the hydroxyl ions will be less repelled and oxidation favored. The above results show that such a result is produced by radiant energy. Since it is well known that practically all organic matter, living or dead, carries a negative charge, and since the oxidation of all organic matter is greatly accelerated by radiant energy, it would seem that this theory might find quite wide application.

It was thought interesting in this connection to determine what might be the effect of illumination upon the rate of wandering of bacteria in the electric current. *Sarcina flava*, *Sarcina rosea* and *Bacillus prodigiosus* were investigated. The rates of wandering were first determined in the dark, and then in the light. The results were in all cases positive. In every case the rate of wandering was less in light than the dark. The difference was in general about twenty per cent. We have here a possible electrical explanation of the toxic effect of sunlight on bacteria. Bacteria live best in the dark. If we assume that they take on such a charge as regulates the rate of oxidation to such a value as is demanded by the normal metabolism, then anything which reduced this charge would tend toward destructive oxidation. Radiant energy is capable of reducing the negative potential of the bacteria, and the result is clear.

Another class of phenomena which receives ready explanation from this point of view is that of the coagulation of many proteins

under the influence of ultra-violet rays. It is generally considered that the coagulation of colloids is favored by the reduction of the electric field between the colloid particle and the medium in which it exists. As has been shown radiant energy in general affects the field and very commonly reduces it, which would explain the coagulation in such cases.

In a purely tentative and speculative way, it may perhaps be justifiable to raise the question as to what extent the above principle may be applied to the explanation of any or all of the life processes. From the physical-chemical point of view it is not unreasonable to assume that all metabolic processes which take place between a colloid particle and the medium in which it is found are determined and regulated by the establishment of certain potential differences between colloid and medium. There will be in any particular case, such a potential developed as is most favorable to the particular work in hand. So long as such favorable conditions of potential are maintained metabolism will proceed smoothly. Such influences as disturb the potential system will disturb the metabolism, and such disturbance may be either favorable or unfavorable. It may be going too far, but is it not possible there is some suggestive value in the assumption that living and dead matter are to be distinguished by the ability or inability to maintain such potential systems as properly regulate the metabolic processes? Certainly there would seem to be here a possible explanation of the fact that the living stomach does not digest itself, while the dead one does.

It is also interesting to note that bacteria, whose metabolism is essentially one of oxidation, and which are adjusted to the dark, have their potentials so affected by light as to lead to destruction. On the other hand, chlorophyll, whose function is that of an agent in a metabolism which is essentially reducing, and which is active only in the light, has its potential so affected by light as to favor its type of metabolic reaction. In other words the charge on bacteria is reduced by light, that on chlorophyll is increased.

The author desires that the above be considered not as the exposition of a theory, but merely as the suggestion of one.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Fifty-fourth meeting.

Zoological Laboratory, Columbia University, May 21, 1913.
President Ewing in the chair.

98 (794)

The color index and color of the red blood corpuscles.

By **E. E. Butterfield.**

[*From the Rockefeller Institute for Medical Research.*]

The present study is based largely on material observed several years ago in Munich in the clinic of Prof. Friedrich Müller. The object of the study was to determine by means of exact methods, (1) the existence of a high color index in pernicious anemia, (2) the magnitude of the elevation of the color index, and (3) the explanation of the phenomenon.

The calibration of the pipette and the dimensions of the counting chamber used for the erythrocyte counts were checked by special methods. The hemoglobin determinations were made spectrophotometrically (spectrophotometer of König, Martens and Grünbaum, Nernst filament as light source). The manner in which the hemoglobin concentration may be calculated from a measurement of the light absorption follows from the equation for the diminution in the intensity of homogeneous light on traversing a planparallel layer of a colored solution:

$$I' = Ie^{-klc}, \qquad (1)$$

in which I = initial intensity, I' = final intensity, k = a constant, l = linear thickness of the absorbing layer, and c = the concentration of the colored substance. I'/I can be measured with spectro-

photometer. When this has been done with a solution of pure oxyhemoglobin of known concentration in an absorption tube of known length k can then be calculated. After the value of k has been determined for pure oxyhemoglobin from human blood the concentration of hemoglobin in laked human blood can be derived from a measurement of I'/I. Oxyhemoglobin from ox blood may be conveniently substituted for human hemoblogin in the determination of k since it was shown in Tübingen that the constant has the same value for both hemoglobins. In the present study homogeneous light was not employed, but the measurements were made in a very narrow interval in the spectrum (about 4 $\mu\mu$). This is not accompanied by an appreciable error as long as the concentration and linear thickness are kept within narrow limits. The measurements were always made in two regions in the spectrum, (1) at the maximum in green of the second absorption band of oxyhemoglobin, and (2) at the minimum in yellowish green between the two absorption bands of oxyhemoglobin. The reason for this follows on combining the two equations for these measurements. If $I_1' = I_1 e^{-k_1 l c}$ represent the conditions in the first region and $I_2' = I_2 e^{-k_2 l c}$ those in the second region, one would have after logarithmating and dividing,

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{k_1}{k_2} = K. \qquad (2)$$

Therefore

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

remains constant independent of concentration and linear thickness. If

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

varies then some other substance with constants different from k_1

and k_2 must also be present in the solution. For pure oxyhemoglobin $k_1 = 19.36$ and $k_2 = 11.66$. As concentration grams in 100 c.c. is used. The blood was taken in all cases from an arm vein and immediately defibrinated. Table I gives the result of determinations made chiefly on individuals in apparent good health and on patients with pernicious anemia.

TABLE I.

NORMAL BLOOD.

	Specific gravity.	Sahli reading.	Erythrocytes. Millions per cu.mm.	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$	Hemoglobin content of blood. Grams per 100 c.c.	Quantity of hemoglobin in average erythrocyte. Grams $\times 10^{-11}$.	Color index.
Men, 1	1.057	95	4.29	1.66	15.4	3.6	1.1
2	1.055	77	4.19	1.65	14.3	3.4	1.0
3	1.062	100	5.19	1.67	17.4	3.2	0.9
4	1.061	97	5.46	1.68	17.8	3.3	1.0
5	1.056	87	4.57	1.64	15.3	3.4	1.0
6	1.066	105	5.79	1.65	19.3	3.3	1.0
7	1.060	93	4.74	1.65	16.9	3.6	1.1
Averages	1.059	93	4.92	1.66	16.6	3.4	1.0
Women, 1	1.057	83	4.42	1.64	15.8	3.6	1.1
2	1.058	88	5.11	1.64	15.6	3.1	1.0
3	1.053	77	4.44	1.67	13.7	3.1	1.0
4	1.061	97	5.11	1.64	17.0	3.3	1.0
5	1.055	85	4.46	1.64	13.7	3.1	1.0
6	1.056	90	4.95	1.65	15.2	3.1	1.0
Averages	1.057	87	4.75	1.65	15.2	3.2	1.0

PATHOLOGICAL CASES.

Pernicious anemia I.	1.040	21	0.74	1.65	3.47	4.7	1.5
Pernicious anemia II.	1.035	23	0.87	1.65	3.79	4.4	1.3
Secondary anemia	—	—	2.43	1.63	5.59	2.3	0.7
Polycythemia	1.075	—	—	1.66	23.9	—	—

It will be seen first that the average hemoglobin content of defibrinated normal blood is considerably higher than the orthodox 14 grams in 100 c.c. Secondly, the value for the amount of hemoglobin per corpuscle in normal blood is remarkably constant. This may be taken as a measure of the color index, and the color index may be conveniently defined as the quantity of hemoglobin per corpuscle multiplied by that factor which renders the quantity of hemoglobin per normal corpuscle = 1. In pernicious anemia

there is a marked increase in the quantity of hemoglobin per corpuscle. That this is an actual increase and not a simulated effect due to the presence of some substance with a greater light absorption than oxyhemoglobin is shown by the constant value for

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

in normal blood and in the blood of pernicious anemia.

Closely related to the color index is the actual color of the red blood corpuscles. It is well known that the color of erythrocytes in single layer viewed microscopically in transmitted light is yellowish green. The blood itself is deep red in color, and the reddish tinge becomes noticeable microscopically when several superposed corpuscles are viewed in transmitted light. The explanation of this phenomenon becomes apparent, I think, when one studies the spectrum of a single layer of corpuscles as compared with several superposed layers. The absorption curve of oxyhemoglobin presents a minimum in yellowish green (560 $\mu\mu$) and a region of least absorption in red (650 $\mu\mu$–660 $\mu\mu$). In the oxyhemoglobin spectrum k_{gr} is much greater than k_r, consequently on increasing the thickness of the absorbing layer the intensity of the transmitted light diminishes much more rapidly in yellowish green than in red. If we regard only these two regions in the spectrum we would have for the intensity of the transmitted light in yellowish green $I_{gr}' = I_{gr}e^{-k_{gr}l}$ and in red $I_r' = I_r e^{-k_r l}$. For $I_{gr}e^{-k_{gr}l} > I_r e^{-k_r l}$ a color change would be expected. This is known as the principle of dichromatism. Whether a color change occurs or not with the same light source would depend on the values of k_{gr} and k_r and on l. The necessary conditions $I_{gr} > I_r$, $k_{gr} > k_r$ for oxyhemoglobin and l sufficiently small are all realized in the case of red blood corpuscles viewed in daylight. This formulation is only a very rough approximation. The exact formulation would require integration of the intensities over the whole spectrum, and this cannot be done at present as long as it is not known what functions I and k are of λ (the wave-length). However, several observations furnish strong support for the view that the color

change of erythrocytes in layers of varying thickness is in accordance with the principle of dichromatism. It is possible to construct a thin wedge of solid oxyhemoglobin and observe the thickness at which the color change occurs. In such a wedge at a thickness of 1.3 μ and less the color is identical with that of a single layer of red blood corpuscles. Above 1.3 μ a distinct reddish tinge is noticeable, increasing with the thickness of the wedge to a deep pure red. In this experiment the color is the same in parallel or convergent light. This rules out the influence of the stroma and the surface curvature on the color of the red blood corpuscles. Finally, small (microscopic) crystals of oxyhemoglobin (second crystallization) are of the same color as the red blood corpuscles, while larger (*i. e.*, thicker) crystals are bright red.

99 (795)

Combined action of magnesium and ether; evidence of a central effect of magnesium.

By **S. J. MELTZER** and **JOHN AUER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

We have shown about eight years ago that magnesium sulphate is capable of causing a profound depression in animals. After an injection of a proper dose of a solution of a magnesium salt the animal loses for some time, all reflexes and signs of sensibility, while the respiration remains intact. Several years before it was found (M.) that a condition similar to this can be produced by an intra-cerebral injection of two or three drops of a 5 per cent. solution of magnesium sulphate, while the injection of hypertonic solutions of other salts caused convulsions. On the basis of both experiences we assumed as a working hypothesis that magnesium favors an inhibition of the entire nervous system. We designated the depressed condition of the animals as anesthesia, which implied that the central nervous system was also affected. This interpretation has not been accepted by Wiki. He called attention to experiments of Binet and of his own to the effect that magnesium salts paralyze the motor nerve endings, and he assumed that in our

experiments the animals were merely paralyzed and had not lost any sensation; in short, magnesium acts, according to Wiki, like curare, although he admits the significant difference that curare paralyzes the respiratory motor nerves before the motor nerves of the other parts of the body, while magnesium paralyzes all other motor nerves before it attacks the motor nerves concerned in the respiration. The statement that magnesium paralyzes motor nerve endings is perfectly correct; we have seen it ourselves numerous times. While it is true that many other inorganic salts have also a curare-like action upon the motor nerve endings, it has to be admitted that the effect of magnesium salts upon the motor nerve endings exceeds that of any of the other salts. This fact, however, is rather in harmony with our assumption that magnesium depresses all parts of the nervous system. The question is only whether it affects also the central part of the nervous system. Wiki and two or three others deny it; we assume it, and have many good reasons for this assumption. We shall, however, not enter here upon a discussion of the entire subject. Our sole purpose in the present communication is to report the results of a series of experiments which make it probable that magnesium affects also the central nervous system. In these experiments rabbits and dogs received one half, or less, of the effective dose of magnesium sulphate. It was found that such animals are readily deeply narcotized by inhalations of small doses of ether which are insufficient to narcotize normal animals. You see here a picture of three rabbits. The dose of magnesium sulphate necessary to narcotize a rabbit is about 1.2 gm. per kilo body weight. Rabbits No. 1 and 3 each received intramuscularly 0.6 gm. $MgSO_4$ per kilo. Rabbits No. 2 and 3 inhaled through tracheotomy tubes, connected by means of a T-tube with the tube of a bottle containing ether. Each rabbit received exactly the same amount of ether which was insufficient to cause complete narcosis. The animals were photographed soon after the discontinuation of the etherization. Rabbit No. 1 which had only magnesium, and No. 2 which had only ether are sitting up. Rabbit No. 3 which had magnesium and ether is deeply narcotized and is limp. You see here a similar picture of three dogs treated in the same manner. If magnesium would have had only a peripheral effect there could

have been no summation with the subminimum central effect of the ether.

100 (796)

Note on the production of acid by tissues growing in vitro.

By PEYTON ROUS, M.D.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

Connective-tissue cells of the chicken, growing *in vitro* in chicken plasma to which a little blue litmus has been added, produce rapidly a focal, pink coloration of the medium. If a number of small fragments of one tissue (heart muscle or the aorta of young chicks, or chicken sarcoma) be plated out with the plasma medium in a petrie dish, it will be found that all the tissue bits are at first stained blue, but that those from which growth occurs become pink, while the growing tissue itself is unstained. The fragments remaining permanently inert keep the blue color.

Often a pink coloration of tissue bits can be observed at a time when growth is found, microscopically, to have barely started. The acid change is in general sharply localized to the neighborhood of the growing tissue. When growth is checked by placing the preparation in the ice-box, neutralization in the acid foci is often incomplete at the end of forty-eight hours, and this even when the bulk of alkaline plasma is relatively large and its plasma network thinned by dilution. Diffusion in the plasma medium as thus indicated is very slow. Under the ordinary circumstance of *in vitro* life without artificial provision for a circulation of fluid, tissue proliferation must take place almost from its beginning, in an acid medium. This constitutes a serious fault in the method of cultivation.

The nature of the acids produced by the growing tissue has not been determined. Carbonic and lactic acids are presumably present in greatest quantity. That the amount of acid formed may be very considerable has been shown by titrating out the as yet unclotted blue plasma to the tint acquired by the tissue cultures. The acid does not affect methyl orange, but very occasionally it changes congo red toward violet, a change best seen

in the interior of degenerating cells which have taken up the indicator. The violet change is not due to free carbon dioxide, for bubbling the gas through unclotted plasma containing congo red fails to bring about an alteration in color.

The observations show that tissue cells can withstand much more considerable changes in the reaction of the medium round about than has been supposed; and that their growth *in vitro* may be very active in an acid medium.

101 (797)

The location of the optic anlage in Amblystoma and the interpretation of certain eye defects.

By CHARLES R. STOCKARD.

[*From the Department of Anatomy, Cornell Medical College, New York City.*]

The early embryos of *Amblystoma* were operated upon so as to remove definite areas from the open medullary plate in order to determine the position of the materials which would give origin to the future eyes.

Preliminary experiments of sticking and disturbing the cells in the anterior end of the medullary plate without actually removing these cells did not prevent the subsequent development of the optic vesicles in an apparently normal manner. Cutting out rectangular pieces of the anterior part of the medullary plate, reversing the pieces and transplanting them merely caused the eyes to develop in misplaced positions. These two experiments demonstrate the fact that unless the future eye material is well removed by the operation the optic vesicles may form. This fact is important in considering the results of the following experiments.

A general statement of the results of the experiments may be expressed as follows: Thirty embryos studied after various operations in which lateral portions of the medullary plate were removed at slightly different developmental stages showed in twenty-four individuals, or eighty per cent. of the cases, subsequent development of both eyes, while only six specimens, or twenty per cent.

of the cases, showed absence of the eye. In one case the presence of the eye was questionable, in five cases one eye and in one case both eyes were absent. The absence of the eyes in the latter cases was possibly due to the cut having been made in a more median position than was intended.

Nine embryos studied after having been operated upon so as to remove a narrow median strip of cells from the anterior portion of the medullary plate showed in four cases, or about forty-five per cent. of the specimens, entire absence of eyes. In four other individuals the eyes were highly defective, one specimen having one poorly formed eye while the other was questionably present. In only one of the nine embryos did the eyes approach the normal condition, from this specimen an extremely narrow median piece had been cut out of the medullary plate. The optic anlage might have been sufficiently wide at the time of the operation to allow its median portion to be removed and yet enough material remain on either side of the cut to give origin to the two eyes. According to the views of several investigators the removal of this median material should have caused the cyclopean defect, yet it did not. In a more extended report of these experiments I shall show that cyclopia is not due to a coming together of lateral materials in the median plane, but to a failure of median material to spread laterally.

Contrasting the results obtained after the lateral and median cuts mentioned above one must conclude that: *The eye anlage in the medullary plate occupies an antero-median position as shown by the various abnormalities incurred when this region is cut away. The failure to injure the development of the eyes in the great majority of cases when the lateral portions of the medullary plate are removed by operation indicates further that the eye anlagen do not occupy lateral positions during this stage of development.*

Based upon these experiments and a study of a large number of eye abnormalities it is concluded that the cyclopean defect is a developmental arrest. The eye anlage fails to widen laterally so that only a single median growth center arises from which develops the ventro-median cyclopean eye. In normal cases the anlage widens and two more or less lateral growth centers become established and give rise to the ventro-lateral optic

vesicles. The optic stalks, however, and later the optic nerves following the stalks as paths always lead back to the point of their median origin and the optic cross or chiasma is in the median plane, below and outside the brain tissue. The attainment of this position of the optic cross would seem mechanically impossible if the eyes arose from lateral medullary tissues since the optic fibers following the stalks would enter the brain laterally and would necessarily cross within the brain tissue, not below and outside as the nerves actually do.

There is no medullary tissue other than future eye tissue between the eye anlagen, therefore, Spemann and others are incorrect in assuming that cyclopia is due to a failure to develop of tissues between the eyes thus permitting the eye anlagen to slump towards the median plane and fuse. The defect is due to a failure or arrest in development of the eye material itself.

102 (798)

The occurrence of betaine in the muscles of invertebrates.

By D. WRIGHT WILSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

Betaine, or trimethylglycocoll, was isolated from the muscle tissue of two varieties of mollusc, *Pecten irradians*, the common scallop, and *Sycotypus canaliculatus*, the periwinkle.

The tissues used were the adductor muscle of the *Pecten* and the large pedal muscle of the *Sycotypus*. The manner of treatment was the same in both cases. The muscles were finely ground, extracted with several changes of water and the concentrated extract freed of colloidal material by precipitation with alcohol and by the regular Kutscher manipulation with tannin. The portion precipitated by phosphotungstic acid was fractioned by precipitation with silver nitrate and barium hydroxide and from the resulting filtrate, betaine was crystallized as the free base and hydrochloride. In both cases, the compound was identified by the melting points of the hydrochloride, picrate and chloroplatinate and by the analyses of the hydrochloride and chloroplatinate.

103 (799)

Studies in thyroid activity.

I. THE CHEMICAL CONSTITUENTS OF THE THYROID GLAND.

By **E. C. KENDALL.**

[*From the Pathological Department of St. Luke's Hospital, New York City.*]

Interest in the chemical examination of the thyroid gland centers around the iodine-bearing compound. The first attempt made in this laboratory to separate and purify the iodine-bearing compound consisted in dialyzing under varying conditions. The entire gland was dissolved in sodium hydroxide and dialyzed from collodion sacs. With running tap water only ten per cent. of the total iodine would pass through the sac. At a temperature of 60 to 70° C. and under proper conditions of acidity, eighty per cent. of the iodine would pass through the sac. These experiments led to a study of the diffusibility of certain cleavage products of the thyroid. As this study of the chemical constituents of the thyroid is still being carried on, the details will be published later. The results, however, may be briefly described as follows:

A new method of treatment has been found by which the complex proteins of the thyroid gland may be broken down into simpler constituents which have not been described by previous investigators. Among the products obtained uric acid and tryptophan in large amount are found. There are several non-iodine-bearing compounds. The iodine has been found to exist in two distinctly different compounds. These have been partially purified and a compound containing 23.3 per cent. of iodine in organic combination has been separated. Of the non-iodine-bearing compounds two are of especial interest. One reduces silver, gold, and mercury in alkaline solution, and the other absorbs free iodine very readily. In all, twelve distinct chemical compounds have been separated. The properties of three of these show them to be still of high molecular weight and they may, by further treatment, be divided into still simpler compounds.

104 (800)

Studies in thyroid activity.

II. THE SPECIFIC PHYSIOLOGICAL ACTIVITY OF CERTAIN CONSTITUENTS OF THE THYROID GLAND.

By **E. C. KENDALL.**

[*From the Pathological Department of St. Luke's Hospital, New York City.*]

Investigations of the physiological activity of the thyroid gland have shown that the internal secretion of the gland serves many different functions. One or more of a series of symptoms accompany cases of thyroid deficiency. These symptoms are relieved by the administration of the thyroid gland of certain animals, as the sheep, hog, and ox. When this treatment is stopped the symptoms return. It would thus appear that the functions of the internal secretion of the thyroid may be fulfilled by furnishing the body with the constituents of the thyroid gland from another animal.

The separation of the various chemical constituents briefly described above suggested the possibility of determining which constituents controlled the various symptoms occurring in cases of thyroid deficiency.

The first step in the method of separation of the constituents results in two solutions. One of these contains about 60 per cent. of the total iodine and 9 per cent. of the nitrogen. This is designated "Solution *A*." The other, called "Solution *B*," contains 40 per cent. of the total iodine and 91 per cent. of the nitrogen. In order to establish the physiological activity of these solutions, experiments were carried out, first upon dogs, and then with cases of thyroid deficiency. The number of cases treated is insufficient to establish completely the physiological properties of these solutions, but the results based upon a series of experiments with two dogs extending over four months, with two typical cases of myxedema, and with three cretins, are as follows:

Solution *A* was found: (1) to affect the nitrogen metabolism and hence the body weight and temperature; (2) to produce tachycardia; (3) to cause nervousness and tremor; (4) to relieve

pain and great weakness felt in the back and certain sensations of cold felt on the head.

Solution *B* was found: (1) to change the dry, scaly, rough skin to a soft and entirely normal condition, which allowed perspiration to take place; (2) to remove the soreness of bones and joints; (3) to prevent cramps and twitching of the muscles; (4) to have a marked effect on the mental activity, especially noticeable in cretins; (5) to relieve the hard, firm condition of the flesh, allowing it to become soft and pliable; (6) to prevent burning sensations which flash over the skin from one part of the body to another.

Solutions *A* and *B* act independently of each other. Furthermore, each of these solutions has been subdivided and the various constituents separated have continued to act independently. It would thus appear that each function of the thyroid gland is due to the specific physiological activity of the separate and distinct chemical constituents of the gland. Baumann claimed that iodothyrin is the active principle of the thyroid gland. It is evident that there is no one active principle of the thyroid gland, but that there are many, each with its own specific action. The separation of the various constituents makes it possible to treat cases of thyroid deficiency with those portions of the gland whose deficiency is indicated by the symptoms. Furthermore, it will be possible to standardize the active principles of the thyroid gland by a method based on chemical analysis.

I wish to thank Dr. F. C. Wood and the attending physicians of St. Luke's Hospital for the opportunity of carrying on this investigation, and Mr. A. W. Thomas for assistance in the chemical laboratory.

Further work is being carried on, both with the chemical separation and identification of the various constituents and with the physiological activity of the same.

105 (801)

Further studies on muscle creatine.

By **M. S. FINE** and **V. C. MYERS.**

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

In an earlier communication,[1] attention was called to the constancy in the content of muscle creatine for normal animals of a given species, though distinctive for different animals. It was further pointed out that during starvation in the rabbit, the percentage content of muscle creatine may show either an increase or a decrease, the latter depending in considerable part upon the rate and amount of creatine eliminated in the urine.

These observations have been extended to rabbits which have been fed for varying periods upon carbohydrate—without fat or protein. Carbohydrate feeding greatly reduces the elimination of creatine in the urine, as previously observed, though the creatine content of the muscle does not materially differ from that found during a similar length of starvation. In other words, it may be markedly decreased during a long period of carbohydrate feeding. It seems probable that the action of the carbohydrate is simply one phase of the sparing action of carbohydrate on protein metabolism, in this case allowing sufficient time for the body to handle the creatine, *i. e.*, to oxidize it, or change it to creatinine.

That creatine when fed or injected does not reappear in the urine in the form of creatinine, except in traces, or in large amount unchanged, unless given in considerable quantity, has been ascertained by a number of investigators. The possibility that this creatine, which remains unaccounted for, is stored up in the muscle has not been adequately studied. In four experiments on rabbits, the creatine content of the muscle, after repeated subcutaneous injections of creatine, has been found to be uniformly slightly above (4–7 per cent.) the normal amount. This would appear to indicate that a small amount of the injected creatine was deposited in the muscles, though insufficient to account for the creatine not eliminated in the urine either unchanged or in the form of creati-

[1] Myers and Fine, PROC. SOC. EXP. BIOL. AND MED., October, 1912, X, pp. 10 and 12.

nine. In two similar experiments with creatinine, there was apparently a slight increase in the creatine (total creatinine) concentration of the muscle, which we are not prepared to discuss at this time.

The possible influence of autolysis upon the content of muscle creatine and added creatine and creatinine is being investigated.

106 (802)

The total non-protein nitrogen of the blood in nephritis and allied conditions.

By CLIFFORD B. FARR, M.D., and J. HAROLD AUSTIN, M.D.

[*From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia.*]

The following summary covers the results of our study of the non-protein nitrogen of the blood by Folin's methods in a series of fifty-nine hospital patients. Our main concern has been with nephritis but we have examined the blood in many other conditions as opportunity offered.

The patients group themselves into four divisions:

I. Those showing no disturbance of renal function (17 cases).
II. Those with marked cardio-vascular disease of some type, most of which showed urinary changes the result of renal congestion (11 cases).
III. Those showing nephritis (23 cases).
IV. Those in which certain features would lead one to suspect nephritis, but in which the existence of nephritis is not borne out by other findings (8 cases).

Our patients of Group I, suffering from a variety of acute and chronic diseases, but without evidence of disturbance of renal function, showed a total non-protein nitrogen in the blood varying from 16 to 43 milligrams per 100 c.c. From 50 to 60 per cent. of this was in the ammonia-urea fraction. In the patients with cardio-vascular disease with renal congestion, but without evidence of other renal lesion there was no increase of the non-protein nitrogen in the blood, nor alteration of the ammonia-urea percentage, although albuminuria, casts and some impairment of the phenolsulphonephthalein elimination were usually present.

In that type of chronic nephritis characterized by marked albuminuria, cylindruria and edema, there were similar findings. In that type of chronic nephritis associated with hypertension, the non-protein nitrogen was increased, ranging from 40 to 181 milligrams per 100 c.c., and the percentage of the ammonia-urea fraction was usually higher than in non-nephritic cases. The nitrogen values in these patients were subject to rapid fluctuations in the course of a few days and clinical improvement was associated with a fall in the non-protein nitrogen content. Uremia was almost always accompanied by some increase of the non-protein nitrogen in the blood but no constant relation could be established between the degree of the increase and the tendency to uremia.

We believe that this method of estimating the total non-protein nitrogen in the blood is a valuable aid in the clinical study of nephritis and that it can be carried out in any thoroughly equipped clinical laboratory. The error of the method is indicated by the duplicate analyses which were done in almost all cases and which showed an average discrepancy between duplicates of 1.6 milligrams per 100 c.c. of blood. The urea method was in our hands less reliable, and large and inexplicable discrepancies occurred at times in our urea duplicates rendering repetition necessary and causing us to attach less importance to the urea figures.

107 (803)

The toxicity of sodium tartrate with special reference to diet and tolerance.

By W. SALANT and C. S. SMITH.

[*From the U. S. Department of Agriculture, Bureau of Animal Industry, Washington, D. C.*]

The toxicity of the sodium salts of dextro and levo tartaric acid was tested in experiments on frogs and rabbits. Both isomers were found equally toxic in these animals thus contradicting the earlier work of Chabrié[1] on the subject, who claimed that levo was more than twice as toxic as dextro tartaric acid. In experiments on rabbits, diet proved to be an important factor in the determination of resistance to this substance. Animals which were fed oats or oats

[1] *Compt. Rend. Acad. Sc.*, 1893, Vol. 116, p. 1410.

and cabbage succumbed to a dose of 0.4 gm. of the salt per kilo when given by subcutaneous injection. Suppression of urine was usually observed on the first day and death occurred in six to seven days. In starvation, slightly smaller doses were fatal to some rabbits. The resistance was increased considerably when the diet was changed to carrots. Such animals stood 1.0 gm. per kilo by subcutaneous injection, while 1.2–1.5 gm. per kilo were toxic. A moderate degree of tolerance for tartrates was induced in animals which were fed oats and cabbage. By gradually increasing the dose, a large proportion (6 out of 9) of rabbits survived 0.8 gm. per kilo which is twice the fatal dose. Rabbits which were receiving carrots did not acquire tolerance for tartrates. Sodium tartrate was much less toxic when given by mouth. 5 gm. per kilo was found to be the minimum fatal dose.

EXPERIMENTS ON CATS.

Amounts which have been found to be fatal for rabbits did not produce any symptoms in cats. Subcutaneous injection of one gm. per kilo produced a slight diarrhea in some individuals, and had no effect whatever in others. $1\frac{1}{2}$ gm. per kilo proved fatal to one cat but was without action in another. Out of four cats which received 2 gm. per kilo three died, one survived. When sodium tartrate was given by mouth vomiting frequently occurred. In one case, however, when ten gm. per kilo were fed diarrhea was the only effect observed.

108 (804)

The influence of pancreatic and duodenal extracts on the glycosuria and the respiratory metabolism of depancreatized dogs.

By **J. R. MURLIN** and **B. KRAMER.**

[*From the Physiological Laboratory of Cornell University Medical College, New York City.*]

Several dogs completely depancreatized by Hedon's method and eliminating glucose and nitrogen in Minkowski's ratio were treated by intravenous injection of pancreatic extract prepared by Knowlton and Starling's method.[1] The urines collected in twenty-four hour periods exhibited an increase in the D: N ratio

[1] Knowlton and Starling, *Journ. of Physiol.*, 1912, XLV, p. 146.

on the days immediately following. When the urine was collected in short periods after injection, a marked fall in the sugar output was witnessed lasting from four to ten hours but this was followed by a compensating rise which, in some instances, augmented the total for the twenty-four hour period, in others raised it only to the previous level.

Much greater effects were obtained with a double extract of dog's pancreas and duodenal mucosa. The following experiment (No. II) is typical. The effect, however, cannot be ascribed to the organic extract from either the pancreas or the duodenum for the same Ringer's solution in which the tissues were extracted when made alkaline to about the same extent with Na_2CO_3 gave an identical effect.

DOG "Q." OPERATED APRIL 17.

Exp. II.

Date.	Time.	Vol. urine, c.c.	Glucose, gm.	Nitrogen, gm.	D:N.	Glucose per hour.	Nitrogen per hour.	Blood sugar, per cent.
4/21/13	12.15– 2.15	30	2.41	0.95	2.52	1.20	0.47	0.128
	2.15– 4.15	50	2.40	1.09	2.20	1.20	0.54	
	5.00– 5.30	150 c.c. of pancreas and duodenum from 2 normal dogs inj. intravenously						
	4.15– 6.15	30	1.30	0.67	1.93	0.65	0.35	
	6.15– 7.15	25	0.28	0.33	0.87	0.28	0.33	
	7.15– 8.15	26	0.41	0.45	0.89	0.41	0.45	0.148
	8.15– 9.15	26	0.59	0.48	1.21	0.59	0.48	
	9.15–10.15 P.M.	22	0.51	0.39	1.28	0.51	0.39	
4/22/13	10.15– 6.00		18.66	6.99	2.68	0.93	0.35	
			Exp. III.					
4/24/13	2.19–4.19	22	1.65	0.54	3.08	0.82	0.27	
	4.26–5.15	150 c.c. Ringer's Sol. +1% Na_2CO_3 injected intravenously						
	4.19–6.19	60	1.65	0.64	2.55	0.82	0.32	
	6.19–8.19 A.M.	24	0.46	0.52	0.88	0.23	0.26	
4/24–25	8.19–1.19	90	2.41	0.59	1.62	0.48	0.29	

The similarity in these two experiments on the same dog indicates that it is not a hormone which is responsible for the reduction in the sugar, and the increase in the percentage of sugar in the blood indicates that the reduced sugar elimination is in reality due to a change in the permeability of the kidney, as Wohlgemuth[2] has found it after ligation of the pancreatic ducts.

That there is no effect on the combustion of sugar attending

[2] Wohlgemuth, *Berl. klin. Woch.*, February 24, 1913, p. 339.

the reduced elimination of that substance is proved by the following experiment on another dog place in the respiration calorimeter.

DOG "*S.*" OPERATED May 6.

Date.	Time.	Glucose in urine per hour.	Nitrogen in urine per hour.	D:N.	CO_2, gm.	O_2, mg.	R. Q.	Heat produced.	Temp. of dog.
5/9/13	9.45–10.45				8.62	9.22	0.68	21.73	38.6
	10.45–11.45				9.97	10.55	0.69	27.12	38.6
	8.50–11.55 P.M.	1.74	0.56	3.08					
	12.35– 1.05	150 c.c. exts. of pancreas and duodenum from 2 normal dogs injected intravenously. 20 gms. glucose given per os.							
	11.55– 1.05	1.07	0.39	2.70					
	1.55– 2.55				13.60	14.13	0.70		39.6
	2.55– 3.55				11.84	11.78	0.73		
	3.55– 4.55				10.16	10.71	0.69		39.9
	1.05– 5.05		0.45						

Already in the first hour's urine, including the period of injection, a marked decline in the sugar elimination is seen, showing that the typical effect on the excretion of sugar would have been produced, if glucose had not been fed. With 20 grams of glucose available, however, none, or an extremely small quantity at the most, was burned. The increase in respiratory metabolism the first two hours was due to restlessness of the dog.

Other experiments after injection of pancreatic extract alone and after injection of normal dog's blood likewise showed no effect on the respiratory quotient.

Incidentally it has been found in the single experiment in the respiration calorimeter that the heat production in the depancreatized dog was from 30 to 50 per cent. higher than the normal on the same dog determined one month earlier. This confirms the observations of Benedict and Joslin in severe cases of human diabetes.[1]

[1] Benedict and Joslin, Carnegie Institution of Washington Publication No. 176.

109 (805)

The carbon dioxide and oxygen content of the blood after ligation of the abdominal aorta and the inferior vena cava.

By J. R. MURLIN, L. EDELMANN and R. C. GILES.

[*From the Physiological Laboratory of Cornell University Medical College, New York City.*]

Respiration experiments on normal and depancreatized anaesthetized animals after ligation of the abdominal aorta and the inferior vena cava[1] and on normal animals after exclusion of the liver by joining the portal vein to the inferior vena cava[2] show an increase in the respiratory quotient, which is interpreted by the v. Noorden school to demonstrate the combustion of sugar in the depancreatized animal and the dependence of the normal animal upon the liver for its ability to burn protein and fat. These experiments, however, were not accompanied by analyses of the blood gases. It is possible that the higher respiratory quotient after shortening of the circulating stream might be due to an interference with the oxygen absorption (passive congestion of the lungs) or to increased elimination of carbon dioxide by more rapid circulation of the blood through the lungs.

Preliminary to some respiration experiments on depancreatized dogs in which we are seeking the explanation of the altered respiratory quotient, we have made a number of experiments on normal dogs analyzing the carotid blood before and after simultaneous clamping of the abdominal aorta and the inferior vena cava. The results follow:

Date.	Dog No.	Wt., kgm.	Pulse per min.		Resp. per min.		O_2 per cent. in blood.		CO_2 per cent. in blood.		Clamps on.
			Normal.	Clamped.	Normal.	Clamped.	Normal.	Clamped.	Normal.	Clamped.	
1913											
3/22	I	8	108	140	36	30	14.73	14.83	43.16	22.53	30 min.
4/10	II	12	66	102	35	12	16.40	16.45	38.35	37.47	23 min.
4/19	III	9	132	121	66	77	18.88	16.39	39.42	24.28	1 hr.
5/10	IV	10	108	120	33	15	12.62	10.90	57.06	34.16	1 hr.

[1] Porjes, O., *Biochem. Zeitschr.*, 1910, XXVII, p. 131; Porjes and Salomon, *idem*, p. 143.

[2] Verzar, *idem*, 1912, XXXIV, p. 52.

Chloretone anaesthesia was used. In the case of the last dog which eliminated about 80 c.c. of CO_2 per minute, it may be estimated that a reduction from 57 to 34 per cent. would remove from the body in the course of one hour, about 800 c.c. of CO_2 or enough to raise the R. Q. from 0.75 to 0.88. It cannot be assumed as Porges has done that the blood gases, under the circumstances, would reach an equilibrium within 10–15 minutes, for with the circulation diminished to one half and the heart beating at its normal rate, or higher, the blood would pour through the lungs twice as often and would continue to lose carbon dioxide until the tension in all the tissues became very much reduced.

In dog II the compensation in the rate of respiration was almost sufficient to prevent loss of CO_2.

110 (806)

On the influence of certain diets upon the growth of experimental tumors.

By **J. E. SWEET, E. P. CORSON-WHITE** and **G. J. SAXON.**
[*From the Laboratory of the American Oncologic Hospital, Philadelphia, Pa.*]

The study of the experimental tumors of animals has brought forward numerous interesting observations upon the variation in susceptibility of animals of the same species obtained from different sources, to a given tumor strain, as well as variation in the rate of growth of the transplanted tumors. We have undertaken the study of the relation of certain diets to tumor growth and wish to briefly report the results obtained with a diet based upon the work of Mendel and Osborne. In their studies of the effect of feeding with pure vegetable proteins they encountered numerous combinations which effectively prevented growth, the animal meanwhile appearing in good health. This seemed to us to offer a most interesting opportunity to study the behavior of the tumor cell under these conditions; in other words, regardless of whatever the cause of cancer may be, can an inoculable tumor grow in a host which is apparently incapable of normal cell growth?

This report, while based on a small series as tumor experiments go, shows a result so uniform and striking that its con-

sideration would seem justified. In these series we have made use of white mice, having by preliminary observations determined that a diet made up on the basis of Mendel and Osborne's work, of a combination of glutenin and gliadin, would effectively retard the growth of young white mice. One series of fifty mice inoculated with the tumor obtained through the courtesy of Dr. Rous, of the Rockefeller Institute, gave twenty-three tumors out of twenty-five mice on a normal control diet, but only four out of twenty-five on a vegetable protein diet, of which three tumors later disappeared. In another series of fifty males, all again inoculated with the same tumor, eighteen out of twenty-five on normal diet developed tumors, with three out of twenty-five on a vegetable protein diet; a third series of fifty females gave fifteen tumors out of twenty-five on normal diet with seven out of twenty-five on a vegetable protein diet. Expressed in percentage, 75 per cent. of seventy-five mice developed a tumor under normal conditions; 19 per cent. of seventy-five mice developed a tumor when fed on a vegetable protein diet, and further the tumors in the latter series at thirty days were hardly larger than the tumors in the normal fed mice at ten days.

By referring to the work of Mendel and Osborne it will be seen that it is not a question of starvation in the ordinary sense of the word nor of anemia, but that the most probable conclusion is that the tumor cell is subject to the same laws of growth as is the normal somatic cell.

III (807)

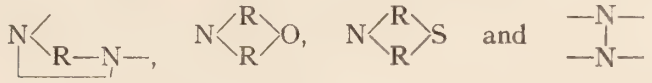
On the inhibitory action of certain anilin dyes upon bacterial development.

By CHARLES E. SIMON, B.A., M.D., assisted by MARTHA A. WOOD, M.D.

The triamino triphenyl methanes possess a well-marked inhibitory power over the development of certain pathogenic organisms, notably staphylococci, streptococci, pneumococci and meningococci, besides the anthrax bacillus and actinomyces. This is quite pronounced, even in a dilution of 1 : 100,000. The common pathogenic *bacilli* are not affected by the dyes in question, in this concentration.

The inhibitory effect is referable to the underlying chromophoric group, and the presence of the basic auxochromic groups. If the basicity of the dye is diminished by the replacement of the basic by acid auxochromic groups, or if such groups are introduced in addition to the basic groups, the inhibitory effect is destroyed.

Of the other classes of anilin dyes an inhibitory effect is produced only by those which contain the chromophonic radicles:



and here also, only by those which contain basic auxochromic radicles. Acid dyes possess no inhibitory properties. The most active inhibitory dyes are the triamino triphenyl methanes, while the indamins, the oxazins, the thiazins and azins are on the whole less active in this respect.

The inhibitory effect does not depend upon the color of the dye, as there are violet acid dyes which are non-inhibitory, and red basic dyes which are markedly inhibitory; similarly there are green dyes which are active and others which are inactive in this respect. The essential common factor evidently is the absence of acid and the presence of basic auxochromic groups. Not all representatives of the sensitive groups of organisms are equally influenced by the dyes in question, nor even by a single dye, nor are the different groups affected to the same extent. Certain dyes will inhibit the growth of all the different sensitive groups, while the effect of others is less extensive.

The inhibitory action of the dyes in question is most readily explained on the assumption that the susceptible organisms combine with the dyes by means of corresponding nutriceptors, but are unable to cause the cleavage of the anchored molecules, the death of the organism resulting from interference with its normal nutritional (sc. reproductive) functions. Upon this basis a directly toxic effect on the part of the dye need not be assumed. It is in accord with the validity of this hypothesis that dye-resistant strains of susceptable groups exist in nature as such, or may be produced artificially. This was accomplished in the case of certain hay bacilli, staphylococci and streptococci. Upon this

basis adaptation of this order would be due to the production of receptors different from those found in dye susceptible strains, by which the nutrition (sc. reproduction) of the organism could be carried on, and which either possesses no affinity for the dye in question or through which the organism can bring about its cleavage. A limited number of observations would suggest that in the treatment of certain infections, with staphylococci and streptococci more especially, certain dyes might be used to advantage. In two cases of erysipelas the repeated local application of the concentrated solution of dahlia seemed to restrict the extension of the infection.

112 (808)

Waxy degeneration of muscle in venom intoxication.

By RICHARD M. PEARCE, M.D.

[*From the John Herr Musser Department of Research Medicine University of Pennsylvania, Philadelphia.*]

In connection with the discussion by Beneke[1] and Wells[2] of waxy degeneration of muscle occurring in anaphylactic poisoning, and especially in view of Beneke's reference to the action of " brasilianischen Schlangengift " (*Crotalus terrificus*) the following notes on waxy degeneration in the rabbit following the intravenous injection of the venom of *Crotalus adamanteus* may be of interest.

In the course of three series[3] of experiments on a total of about 45 rabbits it was noted that the intravenous injection of venom was followed in three instances by waxy degeneration so well-marked as to be evident macroscopically. In two of the

[1] Beneke, R., "Ueber den Kernikterus der Neugeborenen," *Münch. med. Wochenschr.*, 1912, LIX, 387; "Ueber Muskelveränderungen bei akuten Vergiftungen mit Klapperschlangengift sowie bei Anaphylaxie," *Münch. med. Woch.*, 1912, LIX, 729; Beneke, R., and Steinschneider, E., "Zur Kenntnis der anaphylaktischen Giftwirkungen," *Centralb. f. allg. Path. u. path. Anat.*, 1912, XXIII, 529.

[2] Wells, H. Gideon, "Anaphylaxie und Wachsartige Degeneration der Muskeln," *Centralb. f. allg. Path. u. path. Anat.*, 1912, XIII, 945.

[3] Pearce, R. M., "An Experimental Study of the Relative Importance of Renal Injury, Vascular Injury and Plethoric Hydremia in the Production of Edema," *Arch. f. Int. Med.*, 1909, III, 422; "An Experimental Glomerular Lesion Caused by Venom (*Crotalus Adamanteus*), *Jour. Exper. Med.*, 1909, XI, 532; "Experimental Studies of the Influence of Crotalus Venom on the Kidney," *Jour. Exper. Med.*, in press.

animals, attention was called to the lesion by the presence of pale opaque focal area in the psoas mucsle. Microscopical examination of these areas showed that the muscle fibers were irregular in shape, had lost their nuclei and striation and presented the typical hyaline appearance of Zenker's degeneration as it occurs in man. In these animals the adjacent tissue showed no hemorrhage, exudate or evidence of connective tissue reaction. The animals died after 18 and 35 days respectively, the first having received 8 injections and the second 7 injections of venom in doses varying from 0.5 to 2 milligrams.

A third rabbit died on the 38th day after the first injection, having received nine injections, the last on the 23d day. At autopsy mottled hemorrhages were seen in the rectus and psoas muscles and about these hemorrhages, the peculiar opaque, whitish appearance of hyaline degeneration. Upon microscopical examination, the picture was identical with that of true Zenker's degeneration. Irregular, swollen, vacuolated and varicose, hyaline fibers, more or less fractured, without nuclei and invaded by leucocytes occupied large irregular areas. In the midst of these fibers were foci of hemorrhage and throughout an infiltration of polymorphonuclear leucocytes, while about the necrotic areas were wide bands of granulation tissue which sent prolongations between the bundles of muscle fibers. In such areas the surviving fibers frequently showed multiple nuclei.

Whether or not these lesions have fundamentally a common relation with those caused by anaphylactic poisons is of course a matter of doubt. It seems wise, however, to add, in support of Beneke's experience with the venom of *Crotalus terrificus*, these observations on the effect of the venom of *Crotalus adamanteus*.

113 (809)

Note on the effect of animal extracts upon the volume of the thyroid gland.

By ISAAC OTT, M.D., and JOHN C. SCOTT, M.D.

The volume of the thyroid was registered by an oncometer and a modified piston recorder. The arterial tension was also noted. The animals used were dogs, etherized and with a small dose of

morphia per jugular. Infundibulin per jugular had the most powerful action in reducing the volume of the thyroid, although at times there was a preliminary momentary increase. Adrenalin, after a temporary increase, produced a decrement in the volume. An infusion of the fresh ovary of a pregnant cat augmented the size of the thyroid, a fact noted by Hallion. Mammary, corpus luteum, thyroid, placenta, iodine and parathyroid extracts also increased the volume. The anterior part of the pituitary decreased the volume.

114 (810)

The relation of external temperature to hibernation.

By SUTHERLAND SIMPSON.

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

In a former communication[1] to this society it was shown that the absence of food is an important factor in determining the onset of hibernation in the woodchuck (*Marmotta monax*). In the present note attention is drawn to the fact that the cause of the awakening of these animals from their torpid condition in the early spring is not a rise in the temperature of their surroundings.

A colony of woodchucks was kept in artificial burrows a little over four feet[2] below the surface of the ground, as already described.[3] At the bottom of one of these burrows, the oil bulb of a Friez thermograph was placed, and connected with the recording clock-drum contained in a box at the top. All the burrows were packed with dry straw, while the one containing the bulb was shut off from the central court, to prevent the woodchucks having access to it.

A continuous record of the temperature at this depth has been kept from January 1, 1912, till the present time. It shows that the lowest temperature is reached late in March or early in April—just about the time when the hibernating woodchucks are beginning to wake up. There is no appreciable rise in temperature

[1] The Food Factor in Hibernation, PROC. SOC. EXPER. BIOL. AND MED., Vol. 9 (April 17, 1912), p. 92.

[2] Accurate measurement shows the burrows to be a few inches over four feet below the surface and not five feet as formerly stated.

[3] *Loc. cit.*

till well on in April. The diurnal variation, so marked at the surface, is almost completely abolished at this level, all the year round, and this is a circumstance which greatly favors animals with an imperfectly developed heat regulating mechanism, such as the woodchuck possesses.

Records of the air temperature taken at the Ithaca station of the U. S. Weather Bureau, situated about half a mile from the burrows, show that in 1912 the coldest month of the year was January, and in 1913, February, the average mean temperatures for the first four months being as follows:

	Jan.	Feb.	March.	April.
1912	16.3° F.	21.4	28.8	44.5
1913	34.7° F.	23.2	36.8	48.1

Notwithstanding the fact that the weather in these four months was much milder in 1913 than in 1912, the temperature at the depth of four feet, in March and April 1913, as indicated by the thermograph, was about 2° F. lower than in the corresponding months of 1912. The snowfall, however, was greater in 1912 than in 1913 and this will probably explain the apparent anomaly.

	Jan.	Feb.	March.	April.
Snowfall in inches, 1912	9.0	6.7	12.3	0.5
Snowfall in inches, 1913	2.1	5.0	2.9	0.8

In 1912, although the air was intensely cold, the comparatively thick layer of snow effectively retarded the radiation of heat from the surface of the ground.

It is interesting to note, then, that the woodchuck awakes from the hibernating state and becomes active just about the time the temperature of its surroundings has reached the lowest point for the year, and it would appear that some cause other than the temperature or carbon dioxide factor is at work to bring this result about.

115 (811)

Anaphylaxis in immune animals.

By RICHARD WEIL, M.D.

[*From Cornell University Medical School.*]

In his general review of anaphylaxis, Besredka[1] stated that an injection of horse serum into guinea pigs produced hypersensitiza-

[1] Kraus and Levaditi's "Handbook," 1911, p. 248.

tion, only if minute doses were employed. "The preliminary injection of more than .02 cubic centimeter is attended by an uncertain result." Similar statements abound in the literature. The great majority of the experiments on which these conclusions are founded have employed either the intracerebral or the intraperitoneal route for the intoxicating injections. If, however, the second injection is made intravenously, the results are entirely different. With this method, it can be shown that a preliminary injection of as much as five cubic centimeters of horse, or other serum, is invariably followed by a typical anaphylactic state, in which death is produced by the second injection of an amount of the same serum, which, for normal animals, is absolutely innocuous. If guinea pigs are given repeated large injections, *e. g.*, 3 c.c. of horse serum, on three or four successive days, the total amounting to nine or twelve cubic centimeters, exactly the same result follows. Finally, if spaced injections are employed, as in the procedure followed for purposes of immunization, again the animals become typically anaphylactic. There are, however, certain differences in the behavior of animals sensitized, on the one hand, by means of minute, and, on the other, by very massive injections of serum. In the former, the minimal lethal dose is often no greater than .005 cubic centimeter of horse serum. In the latter, it is often one hundred times this amount. Again, after a small injection, anaphylaxis frequently does not develop for three weeks; after massive injections, it may generally be demonstrated in ten or twelve days. These differences are traceable simply to the effects exercised upon antibody production by small, as compared with large doses of antigen. With the former, the antibodies are produced slowly and in small number; with the latter, rapidly and in large number. Thus, in the case of guinea pigs sensitized by small doses, it is generally necessary to use the entire amount of blood obtainable by exsanguination in order to sensitize another normal pig. After large injections, however, one tenth of the total blood may suffice for this result. It is probably on account of the larger amount of circulating antibody that guinea pigs sensitized by large doses require a larger toxic injection.

116 (812)

The parathyroids and pregnancy.

By A. J. CARLSON.

[*From the Hull Physiological Laboratory, University of Chicago.*]

A number of investigators have found, especially in dogs, that pregnancy seems to augment the symptoms of parathyroid tetany, and may transform latent tetany into acute tetany in cases of partial thyroidectomy.[1] The parathyroids must become functional in the fetus a considerable time before birth, because there is no record of parathyroid tetany (acute or latent) in prematurely born infants. In view of the fact that in late pregnancy the pancreas of the fetus seems to be able to compensate for the total extirpation of the pancreas of the mother to such an extent that this operation is not followed by the usual diabetes,[2] an investigation of the other organs of internal secretion with reference to the passage of the hormones from the blood of the fetus to the blood of the mother seemed desirable. Accordingly, in the spring and summer of 1912, thyroid-parathyroidectomy was made in 16 dogs and 11 cats in late pregnancy, that is, within one to three weeks of term.

RESULTS.

1. *Dogs.*—Eight out of the fifteen dogs died in acute tetany within 12 to 24 hours after the parathyroidectomy. In no case was there evidence of labor. Three dogs developed tetany after 36 hours, one dying in tetany on the third day, and the other two on the fourth day without any signs of labor. Two dogs gave birth to the young on the second day and developed tetany symptoms on the third and fourth days respectively. These two died in tetany and depression on the eighth day.

One dog had moderately strong tetany on the second and third days after the operation. On the 4th and 5th days there was no tetany, but one pup was born on each of these days. The dog died in depression on the 6th day. Five pups were found in the uterus. One of the pups was engaged, but the uterine contractions

[1] The literature is reviewed by Biedl, "Innere Sekretion," 1913, I, p. 83.

[2] Carlson and Drennan, *Am. Jour. of Physiol.*, 1911, XXVIII, p. 391.

had evidently been too feeble or incoordinated for the delivery. Two dogs showed no signs of tetany at any time, one of the dogs giving birth to the young on the 6th day, and the other on the 9th day following the parathyroidectomy. Three weeks later the dogs were killed and a search made for accessory parathyroids; none were found.

The development of acute and fatal tetany within 24 hours after parathyroidectomy is very exceptional in male and non-pregnant female dogs. It does occur, however, especially after hemorrhage or prolonged anaesthesia. It would therefore seem that late pregnancy in dogs accelerates and intensifies the parathyroid tetany in the majority of cases. This may, however, be only an addition effect (latent eclampsia + parathyroid tetany).

2. *Cats.*—Ten out of the eleven pregnant cats developed fatal tetany. The slight difference between pregnant and non-pregnant cats is shown by the following comparison:

		Parathyroid tetany; average figures for: 20 males and non-pregnant females.	10 females in late pregnancy.
First tetany symptoms	Maximum	72 hours	48 hours
	Minimum	5 hours	24 hours
	Average	28 hours	30 hours
Death	Maximum	168 hours	175 hours
	Minimum	24 hours	75 hours
	Average	78 hours	120 hours

It is obvious that advanced pregnancy in cats does not accelerate or intensify the symptoms of parathyroid tetany. On the contrary the pregnant cats live a little longer and the excitation symptoms are less severe than in the case of the non-pregnant controls. But the depression seemed greater in the pregnant animals. If the fetal parathyroids are in any way capable of functioning for the mother, this action is so slight that it cannot be demonstrated on the present short series of experimental animals.

It is premature to speculate on the cause of the above difference in the relation of pregnancy to parathyroids in cats and dogs before more data are at hand on other species.

117 (813)

The correlation between the physiological state of the thyroid of the mother and of the fetus.

(PRELIMINARY NOTE.)

By A. J. CARLSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

The starting point of this investigation was some observations (incidental to other work on the thyroids) that pups born of mothers having active hyperplasia of the thyroids seemed to have much larger thyroids than the pups born of mothers with normal thyroids or with colloid goiters. The size of the thyroid in pups from mothers with marked thyroid hyperplasia is in many cases so great that they produce the distortion of the neck similar to goiter in adults. *These pups are apparently born with goiter.*

The work was begun in the spring of 1912, and so far data have been obtained on mother and offsprings in the case of 16 cats and 14 dogs; the work is being continued and extended to other species.

It is well known that goiter (active hyperplasia and colloid) is prevalent in dogs in the Great Lakes region of United States, while in cats in the same region goiter is practically unknown. The goiter of the newborn of mothers with thyroid hyperplasia may be (1) primarily hereditary, that is, due to defects in the germ plasm, or (2) it may be due to some temporary metabolic disturbance in the mother,—toxins or abnormal concentration of normal products of metabolism, acting alike both on the maternal and on the fetal thyroid. If the fetal goiter is due primarily to the constitution of the ovum rather than to the maternal environment during intrauterine life, we would expect the goitre to persist in varying degrees after birth. We would also expect to meet with fetal goiter in the case of mothers with colloid goiters, because the colloid state appears to be preceded by active hyperplasia. On the other hand, if the fetal goiter is due primarily to some intoxication or temporarily altered metabolism of the mother, acting alike on the fetal and on the maternal thyroid so as to produce

hyperplasia, there ought to be no fetal goiters in the case of mothers with normal thyroid or with colloid goiters; and the fetal goiters of mothers with active hyperplasia ought to diminish after birth.

1. *Results in Dogs.*—During intrauterine life the body increases in weight faster than the thyroid gland so that the ratio of the weight of the thyroid to the body weight becomes gradually larger. But in the case of mothers with normal (or nearly normal) thyroids, the ratio of thyroid to body weight is always greater in the mother than in the pups; in the case of mothers with colloid glands the ratio may be greater in the pups than in the mother while in the case of mothers with thyroid hyperplasia the ratio may be the same, or it may be higher or lower, depending on the degree of hyperplasia of the maternal thyroid.

The newborn of mothers with active hyperplasia have invariably much larger thyroids than the pups of normal mothers, but the thyroids of the former do not exhibit any greater degree of differentiation toward adult structure. It would thus seem that the conditions causing thyroid hyperplasia in the mother lead in the fetus to excessive growth of the thyroid rather than to specific thyroid differentiation.

In the case of mothers with colloid goiter the thyroids of the pups are on the whole of the same size as in the pups born of normal mothers. A few thyroid ratios may be cited for illustration.

	Ratio of thyroid to body weight. Mother.	Pups.
V. Normal thyroid	1–6,600	1–3,000
X. Colloid goiter	1–1,300	1–4,200
III. Thyroid hyperplasia	1–1,100	1– 480

On the whole, the relative weight of thyroids in dogs, both adult and newborn, is large in comparison with that in cats. There may be considerable variation in the thyroid:body weight ratio in pups of the same litter. We have not been able to determine whether this is due to primary difference in the ova or to the varying factor of accessory thyroids.

2. *Results in Cats.*—Active hyperplasia or colloid goiter has not yet been found in our pregnant cats. But there is some variations in relative bulk of thyroid tissue. Thus the extremes of thyroid-body weight ratio are 1–4,680, and 1–16,000 respectively.

In general the adult pregnant female weighing 3–3.5 K. has thyroids weighing (fresh) 0.20 g. to 0.30 g. While thyroid hyperplasia has not yet been found in pregnant cats, it is a striking fact that mothers with relatively large thyroids give birth to kittens with relatively large thyroids and *vice versa*. The following figures may be cited as typical:

	Ratio of thyroid to body weight. Mother.	Kittens.
No. X	1–16,250	1–7,326
No. IV	1– 4,680	1–2,000

The results so far point to the following conclusions:

1. Active thyroid hyperplasia is not associated with hypersecretion of the thyroids, because hypersecretion of the thyroids in the mother would retard rather than augment thyroid growth in the fetus.

2. Since fetal goiter is always present in the offsprings of mothers with active thyroid hyperplasia, and never present in the offsprings of mothers with normal thyroids or colloid goiter, it would seem that the fetal goiter is not due primarily to the condition of the germ cells, but to some intoxication of the mother or altered condition of the maternal metabolism. Since the maternal environment acts on the fetus only by the way of the blood, the goiter must be due to substances in the blood acting alike on the fetal and the maternal thyroid to produce cell division and growth, rather than specific thyroid differentiation and secretion. This hyperplasia is therefore not compensatory.

118 (814)

Parathyroid tetany and active immunity.

By A. J. CARLSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Parathyroid tetany in dogs seems to be associated with diminished resistance to bacterial invasion of the mucous membranes, as shown by the frequent infection of the eyes, the nose, and the respiratory passages. This diminished resistance may be due to (1) depression of the processes of active immunity; (2) local de-

pression of the cells of the mucous membranes; (3) disturbance of the body heat regulating mechanism. It is also possible that all three factors are involved.

In order to determine whether the first possibility is a factor, dogs were immunized at varying periods before and after parathyroidectomy, and the influence of the tetany condition on the immunity reaction noted. The particular antibody studied was the lysin developed by the injection of goat erythrocytes into dogs. The immunizing dose consisted of a single intravenous injection of 1 c.c. of a 10 per cent. suspension of goat corpuscles per kilo body weight of dog.

The results so far obtained show that *the active immunity is decreased by the condition of parathyroid tetany.* None of our dogs developed lysin to goat corpuscles to the degree observed in the series of normal dogs reported by Hektoen and Carlson, and by Luckhardt and Becht.[1] The greatest concentration of the specific lysin in our tetany dogs was represented by a serum dilution of 1–8,000; the lowest concentration by a serum dilution of 1–400. Two typical experiments may be cited:

Dog V.	Nov. 1.	No lysis in 1–50.	Complete thyroidectomy.
	Nov. 2.	No lysis in 1–50.	13 c.c. goat corp. susp. inj.
	Nov. 3.	No lysis in 1–50.	No tetany.
	Nov. 4.	No lysis in 1–50.	Strong tremors.
	Nov. 5.	Lysis in 1–50.	No tetany.
	Nov. 6.	Lysis in 1–400.	Tetany.
	Nov. 7.	Lysis in 1–1,600.	Violent tetany.
	Nov. 8.	Lysis in 1–2,000.	Strong tetany.
	Nov. 9.	Lysis in 1–2,000.	Tetany and depression.
	Nov. 10.	Lysis in 1–2,000.	Tremors and depression (died).
Dog VI.	Nov. 2.	No lysis in 1–50.	11 c.c. goat corp. susp. inj.
	Nov. 3.	No lysis in 1–50.	
	Nov. 4.	No lysis in 1–50.	Complete thyroidectomy.
	Nov. 5.	Lysis in 1–50.	Dog seems normal.
	Nov. 6.	Lysis in 1–400.	Tremors.
	Nov. 7.	Lysis in 1–1,000.	Mild tetany.
	Nov. 8.	Lysis in 1–1,600.	Violent tetany.
	Nov. 9.	Lysis in 1–2,000.	Violent tetany, depression.
	Nov. 10.	Lysis in 1–4,000.	Tetany and depression.
	Nov. 11.	Lysis in 1–4,000.	Tremors and depression (died).

[1] Hektoen and Carlson, *Jour. of Inf. Dis.*, 1910, VII, p. 319; Luckhardt and Becht, *Am. Jour. of Physiol.*, 1911, XXVIII, p. 257.

119 (815)

The solubility of certain lead salts in human gastric juice, and its bearing on the hygiene of the lead industries.

By A. J. CARLSON and A. WOELFEL.

[*From the Hull Physiological Laboratory, University of Chicago.*]

1. SOLUBILITY OF WHITE LEADS IN HUMAN GASTRIC JUICE.

WHITE LEAD PAINT DUSTS.

Solubility in pure gastric juice (25 c.c. G.J. to 0.5 gr. lead):
Basic lead carbonate paint dust....46 per cent.
Basic lead sulphate paint dust.... 5.7 per cent.
Solubility in gastric juice+peptone (25 c.c. G.J., 0.1 gr. peptone, 0.5 gr. lead):
Basic lead carbonate paint dust....46 per cent.
Basic lead sulphate paint dust.... 7.3 per cent.
Solubility in gastric juice+milk (G.J. 1–milk 1):
Basic lead carbonate paint dust....none = 0 per cent.
Basic lead sulphate paint dust....none = 0 per cent.
Solubility in 0.5 per cent. HCl (25 c.c. HCl to 0.5 gr. lead):
Basic lead carbonate paint dust....66 per cent.
Basic lead sulphate paint dust.... 6.7 per cent.
Solubility in 0.5 per cent. HCl+milk (HCl 1–milk 1):
Basic lead carbonate paint dust....none = 0 per cent.
Basic lead sulphate paint dust....none = 0 per cent.
Solubility 0.5 per cent. HCl+milk (HCl 2–milk 1):
Basic lead carbonate paint dust....25.4 per cent.
Basic lead sulphate paint dust.... 1.5 per cent.
Solubility in 0.5 per cent. HCl+milk (HCl 4–milk 1):
Basic lead carbonate paint dust....83.5 per cent.
Basic lead sulphate paint dust.... 6.9 per cent.

WHITE LEADS.

Solubility in pure gastric juice (25 c.c. G.J. to 0.5 gr. lead):
Lead carbonate ("Old Dutch Process")....53 per cent.
Basic lead sulphate....25 per cent.
Solubility in gastric juice+peptone (25 c.c. G.J. to 0.5 gr. lead):
Lead carbonate ("Old Dutch Process")....57 per cent.
Basic lead sulphate....27 per cent.
Solubility in pure gastric juice (50 c.c. G.J. to 0.5 gr. lead):
Lead carbonate ("Old Dutch Process")....69 per cent.
Basic lead sulphate....30 per cent.
Solubility in gastric juice+milk (G.J. 4–milk 1):
Lead carbonate....90 per cent.
Basic lead sulphate....34.8 per cent.

2. TOXICITY OF WHITE LEADS WHEN FED TO DOGS AND CATS.

The lead carbonate is much more toxic than the lead sulphate. But both salts produce acute lead poisoning when given in quantities of 0.1 gr. per kilo body weight daily.

3. THE INFLUENCE OF MILK.

When milk and gastric juice is mixed in the proportion of 1–1, the hydrochloric acid of the gastric juice is so completely fixed by the milk proteins or neutralized by the carbonates in the milk that the mixture has virtually no solvent action on the lead salts, but when gastric juice is present in excess the lead salts g. into solution in proportion to the excess of the gastric juice When milk is taken into the stomach there occurs, of course, a similar fixation of the hydrochloric acid, and, in addition, the total quantity of gastric juice is diminished owing to the inhibitory action of the fats in the milk on the processes of secretion.

On the basis of our work we venture to offer these two practical suggestions: (1) The lead carbonate is so much more toxic than the lead sulphate that lead workers as well as the state should aim at the elimination of the use of the carbonate in all industries where this is possible. (2) In addition to taking other important prophylactic measures, the lead workers should drink a glass of milk between meals (say 10 A.M. and 4 P.M.) in order to diminish the chances for any swallowed lead to be dissolved by free hydrochloric acid of the gastric juice, as in some persons there is considerable secretion of gastric juice in the empty stomach.[1]

[1] Hornborg, *Skand. Arch. f. Physiologie*, 1904, XV, p. 209; Carlson, *Am. Jour. of Physiology*, 1912, XXXI, p. 151.

120 (816)

Certain observations on the occurrence of tyrosinase in amphibian egg jell.

By A. M. BANTA and ROSS AIKEN GORTNER.

[*From the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

In experiments having for their object the modification of pigment in amphibian larvae fresh eggs and young embryos of the following species of amphibians were placed in tyrosin solutions of 0.025 per cent. concentration: *Ambystoma punctatum* (L.), *Spelerpes bilineatus* (Green), *Bufo lentiginosus* Shaw, *Hyla pickeringii* Holbrook, *Rana clamitans* Lat. and *Rana sylvatica* Le Conte.

No coloration of the egg membranes or jell appeared in either *Ambystoma* or *Spelerpes*. There were some indications of coloration in *Bufo* jell but the coloration was so slight as to be inconclusive. *Hyla* egg jell showed a pink to violet coloration within 24 hours, after being placed in the solution, and the solution itself took on much the same color. Within 2 or 3 days the jell was much blackened, and the solution was fairly dusky with suspended humin. The egg jell of *Rana clamitans* showed some darkening within a day and after forty-eight hours was conspicuously blackened while the solution was likewise very perceptibly darkened. *Rana sylvatica* egg jell took on the rose coloration within one to three hours after being placed in the solution, and the coloration rapidly proceeded through violet to an intense black so that the eggs were invisible at a depth of 3–4 cm.

These results indicate the presence of tyrosinase in the egg jell of *Hyla pickeringii*, *Rana clamitans* and *Rana sylvatica*, and possibly in *Bufo*, but not in *Ambystoma punctatum* or *Spelerpes bilineatus*.

If the slight evidence of tyrosinase in *Bufo* is taken to indicate its actual occurrence there, tyrosinase is found in the egg jell of four species of Anura but is absent in the two species of Urodela examined. This may possibly prove to be an additional distinguishing character between these two orders of amphibians.

121 (817)

Experiments on the light and tactile reactions of a cave variety and an open water variety of an amphipod species.

By **A. M. BANTA.**

[*From the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

The amphipod, *Eucrangonyx gracilis* (Smith), occurs in the waters of many caves in southern Indiana and is very generally and abundantly distributed in small surface streams in the same region. The individuals occurring in caves almost without exception have very little or no body pigment though possessed of the normal pigment in the eyes. Those living in surface streams have the usual amount of pigment for a crustacean. In other structural characters the forms are apparently alike.

In an attempt to determine whether there was a physiological difference between the two forms they were tested for light and tactile reactions. These amphipods are negative to all intensities of light to which they respond. Extended series of light experiments using horizontal illumination were conducted during which every effort was made to maintain as precise conditions as possible. The cave form was found to be somewhat less responsive to light than the form living outside caves, but this difference was not large and was distinctly observable only with relatively small intensities of light.

In order to test the tactile reactions a delicate camel's hair fastened to the end of a glass rod was used to stimulate various parts of the body of several individuals of the cave form and the same number (of equal size) of the surface form. Complete tabulations of the nature and vigor of each response, when a response occurred, were kept and failures to respond were likewise recorded. This data showed a greater number of failures to respond and on the average a slightly less vigorous response on the part of the surface form as compared with the cave form.

Hence the cave form appears to be less responsive to light and more responsive to tactile stimulation than its outside relative.

RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

VOLUME X.

Atkinson, J. P.

766. [with **C. B. Fitzpatrick.**] On the presence of pressor substances in experimental immunity.

787. [with **C. B. Fitzpatrick.**] On some vaccinia blood pressor substances in rabbits.

Auer, J.

795. See Meltzer, S. J.

Austin, J. H.

802. [with **C. B. Farr.**] The total non-protein nitrogen of the blood in nephritis and allied conditions.

Bancroft, F. W.

776. Heliotropism and galvanotropism in Euglena.

Banta, A. M.

816. [with **R. A. Gortner.**] Certain observations on the occurrence of tyrosinase in amphibian egg jell.

817. Experiments on the light and tactile reactions of a cave variety and open water variety of an amphipod species.

Banzhaf, E. J.

751. See Park, W. H.

Bronfenbrenner, J.

717. See Manwaring, W. H.

743. See Manwaring, W. H.

Burnett, T. C.

788. See Robertson, T. B.

789. See Robertson, T. B.

790. Note on the relation of alexin to thrombin.

Butterfield, E. E.

748. The reaction between oxygen and hemoglobin.

794. The color index and color of the red blood corpuscles.

Calkins, G. N.

742. Further light on the conjugation of paramecium.

Carlson, A. J.

812. The parathyroids and pregnancy.

813. The correlation between the physiological state of the thyroid of the mother and of the foetus.

814. Parathyroid tetany and active immunity.

815. [with **A. Woelfel.**] The solubility of various lead salts in the human gastric juice and its bearing on the hygiene of the lead industries.

Chase, A. F.

758. See MacNeal, W. J.

Clark, P. F.

697. See Flexner, S.

Clowes, G. H. A.

744. Certain specific reactions in hay fever.

745. Treatment of autumnal hay fever by vaccination.

767. [with **F. C. Goldsborough** and **F. West.**] On a complement deviation reaction exhibited in pregnancy.

768. [with **F. C. Goldsborough.**] On the antitryptic reaction exhibited in pregnancy.

Cohn, A. E.

702. On the differences in the effects of stimulation of the two vagus nerves in the dog's heart.

722. The production of reversed cardiac mechanism in the dog.

759. The effects of morphine on the mechanism of the dog's heart after removal of one vagus nerve.

Cohoe, B. A.

785. See Rosenbloom, J.

Cooke, R. A.

724. The fate of parenterally introduced glycogen in human and experimental diabetes.

Corson-White, E. P.

806. See Sweet, J. E.

Crawford, A. C.

739. [with **J. P. Crawford.**] Note on the cock's comb test for the activity of ergot.

Crawford, J. P.

739. See Crawford, A. C.

Dresbach, Melvin.

701. [with **S. A. Munford.**] Interpolated extra-systoles, of frequent occurrence, in an otherwise normal heart.

Du Bois, E. F.

754. See Wiggers, C. J.

Edelmann, L.

805. See Murlin, J. R.

Ewing, E. M.

782. See Janeway, H. H.

Famulener, L. W.

751. See Park, W. H.

Farr, C. B.

802. See Austin, J. H.

Fine, M. S.

703. See Myers, V. C.

704. See Myers, V. C.

801. [with **V. C. Myers.**] Further studies on muscle creatine.

Fitzgerald, J. G.

734. Agglutination of encapsulated bacteria.

735. Relative frequency of B. coli communior in contaminated water.

736. [with **C. L. A. Schmidt.**] Production of creatinine by bacteria.

792. [with **Gertrude Macintosh.**] Action of gentian violet on Mucosus capsulatus group.

Fitzpatrick, C. B.

766. See Atkinson, J. P.

787. See Atkinson, J. P.

Flexner, S.

697 [with **P. F. Clark.**] The mode of infection in experimental poliomyelitis.

Foster, N. B.

719. The effect of phlorhizin on dogs with Eck fistula.

786. Sugar from lactic acid in human diabetes.

Gay, F. P.

772. [with **T. Brailsford Robertson.**] Studies on the nature of biological specificity.

Giles, R. C.

805. See Murlin, J. R.

Githens, T. S.

715. [with **S. J. Meltzer.**] Differences in the toxic effects of ether and chloroform as observed under intratracheal insufflation.

725. Decerebration and the action of morphine in frogs.

760. The influence of temperature on the minimal dose of strychnine in frogs and the time of onset of tetanus.

780. [with **S. J. Meltzer.**] Antitoxic action of sodium iodide on morphine.

Goldfarb, A. J.

755. The influence of the central nervous system in regeneration of the annelid worm.

756. Changes in concentration of sea water and their influence upon regeneration.

784. Experimentally fused larvae.

Goldsborough, F. C.

767. See Clowes, G. H. A.

768. See Clowes, G. H. A.

Haas, S. L.

737. Regeneration of bone from periosteum.

Halstead, W. S.

770. Hypertrophy of the thyroid gland. Revision of experiments made 25 years ago.

771. Partial occlusion of the aorta by bands of living tissue.

Hess, A. F.

750. The pancreatic lipase of infants in acute intestinal disturbances.

Hunter, A.

763. The influence of experimental cretinism upon nitrogenous metabolism in the sheep.

James, Henry.

705. See Williams, H. B.

726. See Williams, H. B.

Janeway, H. H.

782. [with **E. M. Ewing.**] The mechanical factors of excessive artificial respiration and a consideration of their relation to the acapnial theory of shock.

Jordan, H. E.

710. Heterochromosomes in mammals.

727. The intercalated discs of atrophied heart muscle.

Kendall, E. C.

799. The chemical constituents of the thyroid gland.

800. The specific physiological activity of certain constituents of the thyroid gland.

Kleiner, I. S.

720. On elimination through the mucosa of the urinary bladder.

Kramer, B.

706. The rôle of lipoids, and particularly lecithin in narcosis.

804. See Murlin, J. R.

Lambert, R. A.

777. The fat content, morphology and length of life of cells growing in diluted blood plasma.

Macintosh, Gertrude.

792. See Fitzgerald, J. G.

MacNeal, W. J.

758. [with **A. F. Chase.**] Some observations on bacteria of the duodenum.

MacNider, W. deB.

761. The difference in the effect of Gréhant's anesthetic and of morphine-ether on the total output and composition of the urine in normal dogs.

Manwaring, W. H.

698. [with **J. Bronfenbrenner.**] Effects on meningeal tuberculosis of the local injection of foreign leucocytes.

716. On the relation of leucocytic extract to body fluids.

717. [with **J. Bronfenbrenner.**] The Kraus-Hofer phenomenon in tuberculosis.

743. On lysis of tubercle bacilli.

McClendon, J. F.

778. The rate of absorption of water by the skin of the frog, in relation to M. H. Fischer's theory of edema.

779. The dynamics of a model of cell division.

McCrudden, F. H.

699. The importance of calcium for growth.

Meigs, E. B.

781. On the nature of the semipermeable membranes which surround the fibers of striated muscle.

Meltzer, S. J.

712. The effects of strychnine in cardiectomized frogs with destroyed anterior lymph sacs; a demonstration.

713. See Wollstein, Martha.

714. See Wollstein, Martha.

715. See Githens, T. S.

780. See Githens, T. S.

795. [with **J. Auer.**] Combined action of magnesium and ether; evidence for a central effect of magnesium.

Meyer, G. M.

723. See Van Slyke, D.

Morse, Max.

718. The rôle of phagocytosis in involution.

Munford, S. A.

701. See Dresbach, M.

Murlin, J. R.

804. [with **B. Kramer.**] The influence of pancreatic and duodenal extracts on the glycosuria and the respiratory metabolism of depancreatized dogs.

805. [with **L. Edelmann** and **R. C. Giles.**] The carbon dioxide and oxygen content of the blood after ligation of the abdominal aorta and the inferior vena cava.

Murphy, F. T.

721. See Pratt, J. H.

Myers, V. C.

703. [with **M. S. Fine.**] The creatine content of muscle under normal conditions. Its relation to urinary creatinine.

704. The creatine content of muscle during starvation and its relation to urinary creatine.

801. See Fine, M. S.

Ophuls, W.

732. Chronic lead poisoning in guinea pigs. Its relation to polyserositis.

733. Chronic lead poisoning in guinea pigs. Its relation to chronic nephritis.

Oppenheimer, B. S.

753. [with **H. B. Williams.**] Prolonged complete heart block without lesion of the bundle of His with frequent changes in the idio-ventricular complexes.

Ott, Isaac.

708. [with **J. C. Scott.**] The action of glucose upon the amount of epinephrin in the blood.

709. [with **J. C. Scott.**] The inhibitory and synergistic hormones of the secretion of milk.

730. [with **J. C. Scott.**] Note on the action of tonsillar extract.

809. [with **J. C. Scott.**] Note on the effect of animal extracts upon the volume of the thyroid gland.

Park, W. H.

751. [with **L. W. Famulener** and **E. J. Banzhaf.**] Influence of protein concentration upon the absorption of antibodies from subcutaneous tissues.

Pearce, R. M.

746. See Williams, P. F.

808. Waxy degeneration of muscle in venom intoxication.

Pike, F. H.

749. See Wilson, J. G.

Pratt, J. H.

721. [with **F. T. Murphy.**] Pancreatic transplantations in the spleen.

Reichert, E. T.

728. The differentiation and specificity of starches in relation to genera, species, etc.

Robertson, T. B.

738. [with **T. C. Burnett.**] The influence of lecithin and cholesterin upon the growth of tumors.

772. See Gay, F. H.

773. On the nature of Oöcytin; the fertilizing and cytolyzing substance in mammalian blood-sera.

774. On the influence of lecithin upon the development of sea-urchin embryos.

788. [with **T. C. Burnett.**] Preliminary communication on the part played by cholesterol in determining the incidence of carcinoma.

789. [with **T. C. Burnett.**] The influence of digitonin upon the growth of carcinoma.

Robinson, G. Canby.

752. The influence of the vagus nerves on the faradized auricles in the dog's heart.

Rosenbloom, J.

731. The diffusion of iodo-eosin from ether through rubber into ether.

764. See Stetten, DeWitt.

785. Metabolism studies in a case of myotonia atrophica.

Rous, Peyton.

796. Note on the production of acid by tissues growing in vitro.

Rusk, G. Y.

711. A comparison of chemical with micro-chemical methods for the determination of varying amounts of glycogen in the liver.

Salant, W.

803. [with **C. S. Smith.**] The toxicity of sodium tartrate with special reference to diet and tolerance.

Saxon, G. M.

806. See Sweet, J. E.

Schmidt, C. L. A.

736. See Fitzgerald, J. G.

Scott, E. L.

765. The relation of pancreatic extract to the sugar of the blood.

Scott, J. C.

708. See Ott, I.

709. See Ott, I.

730. See Ott, I.

809. See Ott, I.

Sears, H. J.

775. On acid agglutination as a method of differentiation of bacteria.

Simon, C. E.

807. [with **M. A. Wood.**] On the inhibitory action of certain anilin dyes upon bacterial development.

Simpson, Sutherland.

700. The pyramid tract in the porcupine.

729. The motor cortex and pyramid tract in the raccoon.

762. The rate of growth in the dog.

810. The relation of external temperature to hibernation.

Smith, C. S.

803. See Salant, W.

Stetten, DeWitt.

764. [with **J. Rosenbloom.**] Metabolism studies in a case of hypopituitarism, with infantilism of the Lorain type.

Stockard, C. R.

797. The location of the optic anlage in Amblystoma and the interpretation of certain eye defects.

Sweet, J. E.

806. [with **E. P. Corson-White** and **G. M. Saxon.**] The influence of certain diets upon the growth of experimental tumors.

Terry, B. F.

757. Variations in the amount of transformed atoxyl (trypanotoxyl) produced by varying the strength of atoxyl incubated with blood.

Todd, J. L.

783. A note on the transmission of spirochaetes.

Van Slyke, D.

723. [with **G. M. Meyer.**] The distribution of amino acids in the body.

von Adeling, E.

740. An experimental study of poison oak.

Weil, R.

769. On a new factor in anaphylaxis.

811. Anaphylaxis in immune animals.

West, F.

767. See Clowes, G. H. A.

Wiggers, C. J.

754. [with **E. F. DuBois.**] Methods for the production of temporary valvular lesions.

Williams, H. B.

705. [with **Henry James.**] Reversal of the cardiac mechanism.

726. [with **Henry James.**] Reversal of the cardiac mechanism; an additional note.

753. See Oppenheimer, B. S.

Williams, P. F.

746. [with **R. M. Pearce.**] Experience with Abderhalden's test for pregnancy.

Wilson, D. Wright.

798. The occurrence of betaine in the muscles of invertebrates.

Wilson, J. G.

749. [with **F. H. Pike.**] A demonstration of the effects of electrical stimulation of the labyrinth of the ear.

Wollstein, Martha.

713. [with **S. J. Meltzer.**] Pulmonary lesions produced by intrabronchial insufflation of cultures of B. megatherium.

714. [with **S. J. Meltzer.**] The development of experimental pneumonia; a demonstration.

Wood, M. A.

807. See Simon, C. E.

Woodruff, L. L.

747. The kernplasma relation during the life of a pedigreed race of Oxytricha fallax.

Yerkes, R. M.

707. Habit and its relations to the nervous system in the earth worm.

Young, Stewart W.

741. See Zinsser, H.

793. On the influence of light on the electrical potential of bacterial and other suspensions.

Zinsser, H.

741. [with **Stewart Young.**] The failure of union between antigen and precipitin when present in the same serum.

791. On the nature of the union of alexin with specific precipitates.

EXECUTIVE PROCEEDINGS.

Fiftieth meeting.

Cornell University Medical College, October 16, 1912. President Ewing in the chair.

Members present: Auer, Benedict, Brooks, Burrows, Butterfield, Cohn, Eisenbrey, Field, Githens, Goldfarb, Kleiner, Lee, Longcope, McClendon, McCrudden, Manwaring, Murlin, Norris, Pike, Robinson, Senior, Simpson, Swift, Terry, Wadsworth, Wallace, Wiggers, H. B. Williams.

Members elected: E. A. Park, M. Sittenfeld.

Fifty-first meeting.

The Rockefeller Institute for Medical Research, December 18, 1912. President Ewing in the chair.

Members present: Atkinson, Auer, Bancroft, Beebe, Berg, Brooks, Cohn, E. M. Ewing, James Ewing, Field, Fitzpatrick, Gies, Githens, Goldfarb, Hatcher, Kleiner, Lee, Lusk, McCrudden, MacNeal, J. A. Mandel, Manwaring, Meltzer, Norris, Oppenheimer, Pratt, Rous, Senior, Sittenfeld, Swift, Terry, Van Slyke, Wadsworth, Wallace, H. B. Williams.

Members elected: Jacob Bronfenbrenner, P. F. Clark, Melvin Dresbach, Paul J. Hanzlik, Arthur D. Hirschfelder, J. B. Murphy, V. C. Myers, E. L. Scott.

Fifty-second meeting.

College of Physicians and Surgeons, February 19, 1913. President Ewing in the chair.

Members present: Atkinson, Auer, Austin, Bancroft, Beebe, Bronfenbrenner, Butterfield, Calkins, Clowes, Cohn, Cole, Draper, J. W. DuBois, Dunham, Eisenbrey, Elsberg, Emerson, Ewing, J., Famulener, Fitzpatrick, Flexner, Gies, Githens, Goldfarb, Halstead, Harris, Hess, Jackson, Jacobs, Joseph, Kast, Kleiner, Lambert, Lee, Levin, I., Levene, P. A., Longcope, Lusk, Mac-

Callum, W. J., MacNeal, McCrudden, Mandel, J. A., Manwaring, Meltzer, Murphy, Myers, Norris, Oppenheimer, Pappenheimer, Park, Pearce, Pike, Ringer, Robinson, Rous, Scott, Senior, Sittenfeld, Stewart, H. A., Steinhart, Terry, Wadsworth, Wallace, Weil, Wiggins, Williams, Anna, Williams, H. B., Wood.

Members elected: R. A. Cecil, C. Eggleston, G. K. Falk, Davenport Hooker, P. E. Howe, C. J. West.

Officers elected: President, James Ewing; Vice-President, Cyrus W. Field; Treasurer, Charles Norris; Secretary, Holmes C. Jackson.

This was the tenth annual meeting of the Society, permanent organization of which was effected on February 25, 1903. At the conclusion of the regular meeting, the tenth anniversary was celebrated by an informal dinner at the Hofbraü Haus at which an unusually large number of non-resident members was present. The dinner closed with speeches congratulatory in character. The following attended: Auer, Bancroft, Butterfield, Bronfenbrenner, Clowes, Cohn, Draper, DuBois, Dunham, Eisenbrey, Ewing, J., Field, Fitzpatrick, Foster, Gies, Githens, Harris, Hess, Jackson, Jacobs, Janeway, H. H., Joseph, Kast, Kleiner, Lambert, Lee, Levene, Levin, Lieb, Loeb, J., Longcope, Lusk, MacNeal, McCrudden, Mandel, A. R., Mandel, J. A., Manwaring, Meltzer, Meyer, Mosenthal, Murlin, Myers, Norris, Noguchi, Oppenheimer, Pappenheimer, Park, W. H., Pearce, Pike, Ringer, Robinson, Rous, Scott, G. G., Scott, E. L., Senior, Sittenfeld, Stewart, Swift, Terry, Van Slyke, Wadsworth, Wallace, West, Wiggers, Williams, H. B., Wood.

Fifty-third meeting.

University and Bellevue Hospital Medical College, April 16, 1913. President Ewing in the chair.

Members present: Bancroft, Beebe, Bronfenbrenner, Brooks, Butterfield, Eisenbrey, Ewing, E. M., Ewing, J., Field, Fitzpatrick, Githens, Goldfarb, Jackson, Kleiner, Lambert, McClendon, Meigs, Meltzer, Murphy, Norris, Pike, Stockard, Wastenays, Wiggers.

Members elected: A. O. Gettler, J. A. Hartwell, G. G. Scott, Martha Wollstein.

Fifty-fourth meeting.

Zoölogical Department, Columbia University, May 21, 1913. President Ewing in the chair.

Members present: Atkinson, Auer, Austin, Bronfenbrenner, Butterfield, Calkins, Eisenbrey, Ewing, J., Field, Fitzpatrick, Jackson, Kleiner, Meigs, Meltzer, Morgan, Murlin, Myers, Norris, Oppenheimer, Rous, Salant, Scott, E. L., Stockard, Terry, Wiggers, Wilson, Wood.

Members elected: A. H. Caulfeild, J. W. Churchman, H. R. Gaylord, H. H. Janeway, J. R. Mohler, A. R. Moore, R. Pemberton, G. B. White.

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INDEX

OF THE

SCIENTIFIC PROCEEDINGS.

[THE NUMERALS IN THIS INDEX CORRESPOND WITH THE NUMERALS IN PARENTHESIS ABOVE THE TITLES OF THE ABSTRACTS. PAGES ARE NOT INDICATED.]

Abderhalden's test for pregnancy, 746.
Absorption, rate of, of water by skin of frog, 778.
Acid produced by tissues growing in vitro, 796.
Agglutination of bacteria, 734, 775.
Amino acids, distribution of, in body, 723.
Anaphylaxis, new factor in, 769; in immune animals, 811.
Annelid worm, regeneration of, 755.
Anilin dyes, effect upon bacterial growth, 807.
Animal extracts, effect upon thyroid, 809.
Antibodies, protein concentration upon absorption of, 751.
Antigen and precipitin, failure of union between, 741.
Antitoxic action of sodium iodide on morphin, 780.
Antitryptic reaction, in pregnancy, 768.
Aorta, occlusion by living tissue, 771; ligation, effect upon carbon dioxide and oxygen in blood, 805.
Artificial respiration, in relation to shock, mechanical factors of, 782
Atoxyl, 757.
Auricles, vagus nerves on faradized, 752.

B. coli communior, frequency in water, 735.
Bacteria, acid agglutination to differentiate, 775; growth of, effect of anilin dyes on, 807; of the duodenum, 758; production of creatinine by, 736.
Betaine, in invertebrate muscle, 798.
Blood, color of red corpuscles, 794.
Body fluids, relation of leucocytic extract to, 716.
Bone, regeneration of, 737.

Calcium, importance for growth, 699.
Carbon dioxide, in blood after ligation of aorta, 805.
Cardiac mechanism, reversed, 705, 722, 726.
Cardiectomized frogs, effects of strychnine in, 712.
Cell division, dynamics of, 779.
Cholesterin and lecithin, influence upon tumor growth, 738; relation to chronic nephritis, 733.
Color index of red blood corpuscles, 794.
Complement deviation in pregnancy, 767.
Conjugation of paramoecium, 742.
Creatin in muscle, relation to urinary creatinine, 703; during starvation, 704; 801.
Creatinine, production of, by bacteria, 736.
Cretinism, effect upon sheep's nitrogenous metabolism, 763.

Decerebration and action of morphine in frogs, 725.
Diabetes, fate of parenterally introduced glycogen in, 724.
Diffusion of iodo-eosin through rubber, 731.
Dog, growth rate in, 762.
Duodenum, bacteria of, 758.
Eck fistula, phlorhizin on dogs with, 719.
Elimination through the mucosa of urinary bladder, 720.
Embryos, sea-urchin, influence of lecithin upon, 774.
Epinephrin in blood, action of glucose upon, 708.

Ergot, cock's comb test for activity of, 739.
Ether and chloroform, toxic effects, 715.
Extra systoles, in otherwise normal heart, 701.
Eye defects, interpretation of, 797.

Fat content, morphology and length of life of cells in plasma, 777.

Galvanotropism and heliotropism in Euglena, 776.
Gastric juice, solubility of lead salts in, 815.
Glucose and amount of epinephrin in blood, 708.
Glycogen, fate of parenterally introduced, in diabetes, 724; determination of, in liver, 711.
Gréhant's anesthetic and morphine-ether upon dog's urine, 761.
Growth, importance of calcium for, 699; in the dog, 762.
Glycosuria, effect of pancreatic and duodenal extracts upon, in depancreatized dogs, 804.

Habit, relation to nervous system in worm, 707.
Hemoglobin, relation between oxygen and, 748.
Hay fever, specific reactions in, 744; autumnal, treatment by vaccination, 745.
Heart block, complete, without lesion of bundle of His, 753.
Heliotropism and galvanotropism in Euglena, 776.
Heterochromosomes in mammals, 710.
Hibernation, effect of external temperature upon, 810.
Hormones of secretion of milk, 709.
Hypopituitarism, metabolism in, Lorain's type, 764.

Immunity, experimental, pressor substances in, 766; and parathyroid tetany, 814.
Intercalated disks of atrophied heart muscle, 727.
Intestinal disturbances, lipase of infants in, 750.
Intrabronchial insufflation, pulmonary lesions produced by, 713.
Intratracheal insufflation, toxic effects of ether and chloroform under, 715.
Involution, rôle of phagocytosis in, 718.
Iodo-eosin, diffusion of, through rubber, 731.

Kernplasma relation during life, 747.
Kraus-Hofer phenomenon in tuberculosis, 717.

Labyrinth, electrical stimulation of, 749.
Lactic acid, sugar from, in diabetes, 786.
Larvæ, production of grafted, 784.
Lead poisoning, chronic, in guinea pigs, polyserositis in, 732; relation to chronic nephritis, 733; salts, solubility in gastric juice, 815.
Lecithin and cholesterin on tumor growth, 738; influence upon development of embryos, 774.
Length of life, fat content, morphology, of cells in plasma, 777.
Leucocytes, foreign, injection on meningeal tuberculosis, 698.
Leucocytic extracts, relation to body fluids, 716.
Light and tactile reactions in amphipods, 817.
Lipase, pancreatic, in infants in intestinal disturbances, 750.
Lipoids, in narcosis, 706.
Lysis of tubercle bacillus, 743.

Magnesium, central effect of, 795.
Metabolism, cretinism effect upon nitrogenous, in sheep, 763; in case of hypopituitarism—Lorain type, 764; in myotonia atrophica, 785.
Morphin, decerebration and action of, in frogs, 725; -ether and Gréhant's anesthetic upon dog's urine, 761; on dog's heart after removal of one vagus, 759.
Motor cortex and pyramidal tract in raccoon, 729.
Muscle, striated, semi-permeable membranes surrounding, 781; invertebrate, betaine in, 798.
Myotonia atrophica, metabolism in, 785.

Narcosis, lipoids in, 706.
Nitrogen, non-protein, in nephritic blood, 802.

Oöcytin, nature of, 773.
Optic anlage, location of, 797.

Oxygen and hemoglobin, relation between, 748; in blood after ligation of aorta, 805.
Oxytricha fallax, kernplasma, relation in life of, 747.

Pancreas, transplantation in spleen, 721.
Pancreatic extract, relation to blood sugar, 725; effect on glycosuria and respiratory metabolism in depancreatized dogs, 804.
Paramœcium, conjugation of, 742.
Parathyroids and pregnancy, 812; tetany and immunity, 814.
Phagocytosis, rôle of, in involution, 718.
Phlorhizin on dogs with Eck's fistula, 719.
Pneumonia, development of experimental, 714.
Poison oak, study of, 740.
Poliomyelitis, infection in experimental, 697.
Precipitin and antigen, failure of union between, 741.
Pregnancy, Abderhalden's test for, 746; antitryptic reaction exhibited in, 768; complement deviation in, 767; parathyroids and, 812.
Pressor substances in experimental immunity, 766.
Protein concentration and absorption of antibodies, 751.
Pulmonary lesions produced by intrabronchial insufflation, 713.
Pyramidal tract and motor cortex in raccoon, 729; in porcupine, 700.

Regeneration of bone from periosteum, 737; of annelid worm, 755; concentration of sea water upon, 756.
Respiratory metabolism in depancreatic dogs, 804.

Secretion of milk, hormones of, 709.
Semipermeable membranes surrounding striated muscle, 781.
Shock, artificial respiration in relation to acapnial theory of, 782.
Sodium iodide, antitoxic action of, on morphin, 780.
Specificity, biological, studies of, 772.
Spirochætes, transmission of, 783.
Spleen, pancreatic transplantations of, 721.
Starches in relation to genera, species, 728.
Starvation, muscle creatine and urinary creatinine during, 704.
Strychnine, effects of, in cardiectomized frogs, 712; temperature effect upon minimum dose of, 760.
Sugar from lactic acid in diabetes, 786; of blood, relation of pancreatic extracts to, 765.

Tactile reaction, light and, in amphipods, 817.
Tartrate, sodium, toxicity of, 803.
Temperature, effect on dose of strychnine, 760.
Tetanus, temperature effect on onset of, after strychnine, 760.
Thyroid gland, hypertrophy, 770; constituents of, 799; physiological activity of, 800; volume of, effect of animal extracts upon, 809; of mother and of fetus, 813.
Tolerance to sodium tartrate, 803.
Tonsillar extract, action of, 730.
Tubercle bacilli, lysis of, 743.
Tuberculosis, injection of leucocytes on meningeal, 698; Kraus-Hofer phenomenon in, 717.
Tumor growth, lecithin and cholesterin upon, 738; effect of diets upon, 806.
Tyrosinase, in amphibian egg jell, 816.

Urinary bladder, elimination through mucosa of, 720.

Vaccination, treatment of hay fever by, 745.
Vagi, differences in the dog's heart, 702.
Vagus, morphine effects after removal of one, 759; nerves on faradized auricles, 752.
Valvular lesions, production of temporary, 754.
Venom intoxication, waxy muscle degeneration in, 808.

Water absorption, rate of, by skin of frog, 778.
Worm, earth, habit and relation to nervous system, 707.
Waxy degeneration in venom intoxication, 808.

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

FIFTIETH MEETING

CORNELL UNIVERSITY MEDICAL COLLEGE

NEW YORK CITY

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CONTENTS.

S. J. MELTZER: The effects of strychnine in cardiectomized frogs with destroyed anterior lymph sacs; a demonstration. 16 (712).

MARTHA WOLLSTEIN and S. J. MELTZER: Pulmonary lesions produced by intrabronchial insufflation of cultures of B. megatherium. 17 (713).

MARTHA WOLLSTEIN and S. J. MELTZER: The development of experimental pneumonia; a demonstration. 18 (714).

T. S GITHENS and S. J. MELTZER: Differences in the toxic effects of ether and chloroform as observed under intratracheal insufflation. 19 (715).

W. H. MANWARING: On the relation of leucocytic extract to body fluids. 20 (716).

W. H. MANWARING and J. BRONFENBRENNER: The Kraus-Hofer phenomenon in tuberculosis. 21 (717).

MAX MORSE: The role of phagocytosis in involution. 22 (718).

N. B. FOSTER: The effect of phlorhizin on dogs with Eck fistula. 23 (719).

I. S. KLEINER: On elimination through the mucosa of the urinary bladder. 24 (720).

J. H. PRATT and F. T. MURPHY: Pancreatic transplantations in the spleen. 25 (721).

A. E. COHN: The production of reversed cardiac mechanism in the dog. 26 (722).

D. VAN SLYKE and G. M. MEYER: The distribution of amino acids in the body. 27 (723).

R. A. COOKE (by invitation): The fate of parenterally introduced glycogen in human and experimental diabetes. 28 (724).

T. S. GITHENS: Decerebration and the action of morphine in frogs. 29 (725).

H. B. WILLIAMS and HENRY JAMES: Reversal of the cardiac mechanism; an additional note. 30 (726).

H. E. JORDAN: The intercalated discs of atrophied heart muscle. 31 (727).

E. T. REICHERT: The differentiation and specificity of starches in relation to genera, species, etc. 32 (728).

SUTHERLAND SIMPSON: The motor cortex and pyramid tract in the raccoon. 33 (729).

ISAAC OTT and J. C. SCOTT: Note on the action of tonsillar extract. 34 (730).

JACOB ROSENBLOOM: The diffusion of iodo-eosin from ether through rubber into ether. 35 (731).

W. OPHÜLS: Chronic lead poisoning in guinea pigs. Its relation to polyserositis. 36 (732).

W. OPHÜLS: Chronic lead poisoning in guinea pigs. Its relation to chronic nephritis. 37 (733).

J. G. FITZGERALD: Agglutination of encapsulated bacteria. 38 (734).

J. G. FITZGERALD: Relative frequency of B. coli communior in contaminated water. 39 (735).

J. G. FITZGERALD and CARL L. A. SCHMIDT: Production of creatinine by bacteria. 40 (736).

S. L. HAAS (by invitation): Regeneration of bone from periosteum. 41 (737).

T. BRAILSFORD ROBERTSON and T. C. BURNETT: The influence of lecithin and cholesterin upon the growth of tumors. 42 (738).

ALBERT C. CRAWFORD and JAMES P. CRAWFORD: Note on the cock's comb test for the activity of ergot. 43 (739).

EDWARD VON ADELUNG (by invitation): An experimental study of poison oak. 44 (740).

HANS ZINNSER and STEWART YOUNG: The failure of union between antigen and precipitin when present in the same serum. 45 (741).

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FIFTY-SECOND MEETING

PHYSIOLOGICAL LABORATORY
COLLEGE OF PHYSICIANS AND SURGEONS

NEW YORK CITY

FEBRUARY 19, 1913

AND

SECOND MEETING

PACIFIC COAST BRANCH, SAN FRANCISCO, CALIFORNIA

FEBRUARY 1, 1913

VOLUME X

No. 3

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1913

CONTENTS.

GARY N. CALKINS: Further light on the conjugation of paramecium. 46 (742).

W. H. MANWARING and J. BRONFENBRENNER: On lysis of tubercle bacilli. 47 (743).

G. H. A. CLOWES: Certain specific reactions in hay fever. 48 (744).

G. H. A. CLOWES: Treatment of autumnal hay fever by vaccination. 49 (745).

P. F. WILLIAMS and R. M. PEARCE: Experience with Abderhalden's test for pregnancy. 50 (746).

L. L. WOODRUFF: The kernplasma relation during the life of a pedigreed race of Oxytricha fallax. 51 (747).

E. E. BUTTERFIELD: The reaction between oxygen and hemoglobin. 52 (748).

J. GORDON WILSON and F. H. PIKE: A demonstration of the effects of electrical stimulation of the labyrinth of the ear. 53 (749).

A. F. HESS: The pancreatic lipase of infants in acute intestinal disturbances. 54 (750).

W. H. PARK, L. W. FAMULENER and E. J. BANZHAF: Influence of protein concentration upon the absorption of antibodies from subcutaneous tissues. 55 (751).

G. CANBY ROBINSON: The influence of the vagus nerves on the faradized auricles in the dog's heart. 56 (752).

B. S. OPPENHEIMER and H. B. WILLIAMS: Prolonged complete heart block without lesion of the bundle of His with frequent changes in the idio-ventricular complexes. 57 (753).

C. J. WIGGERS and E. F. DUBOIS: Methods for the production of temporary valvular lesions. 58 (754).

A. J. GOLDFARB: The influence of the central nervous system in regeneration of the annelid worm. 59 (755).

A. J. GOLDFARB: Changes in concentration of sea water and their influence upon regeneration. 60 (756).

B. T. TERRY: Variations in the amount of transformed atoxyl (trypanotoxyl) produced by varying the strength of atoxyl incubated with blood. 61 (757).

W. J. MACNEAL and A. F. CHACE: Some observations on bacteria of the duodenum. 62 (758).

A. E. COHN: The effects of morphine on the mechanism of the dog's heart after removal of one vagus nerve. 63 (759).

T. S. GITHENS: The influence of temperature on the minimal dose of strychnine in frogs and the time of onset of tetanus. 64 (760).

WM. DE B. MACNIDER: The difference in the effect of Gréhant's anesthetic and of morphine-ether on the total output and composition of the urine in normal dogs. 65 (761).

SUTHERLAND SIMPSON: The rate of growth in the dog. 66 (762).

ANDREW HUNTER: The influence of experimental cretinism upon nitrogenous metabolism in the sheep. 67 (763).

DEWITT STETTEN and JACOB ROSENBLOOM: Metabolism studies in a case of hypopituitarism, with infantilism of the Lorain type. 68 (764).

E. L. SCOTT: The relation of pancreatic extract to the sugar of the blood. 69 (765).

J. P. ATKINSON and C. B. FITZPATRICK: On the presence of pressor substances in experimental immunity. 70 (766).

G. H. A. CLOWES, FRANCIS C. GOLDSBOROUGH and F. WEST: On a complement deviation reaction exhibited in pregnancy. 71 (767).

G. H. A. CLOWES and FRANCIS C. GOLDSBOROUGH: On the antitryptic reaction exhibited in pregnancy. 72 (768).

RICHARD WEIL: On a new factor in anaphylaxis. 73 (769).

W. S. HALSTEAD: Hypertrophy of the thyroid gland. Revision of experiments made 25 years ago. 74 (770).

W. S. HALSTEAD: Partial occlusion of the aorta by bands of living tissue. 75 (771).

F. P. GAY and T. BRAILSFORD ROBERTSON: Studies on the nature of biological specificity. 76 (772).

T. BRAILSFORD ROBERTSON: On the nature of Oöcytin; the fertilizing and cytolyzing substance in mammalian blood-sera. 77 (773).

T. BRAILSFORD ROBERTSON: On the influence of lecithin upon the development of sea-urchin embryos. 78 (774).

H. J. SEARS (by invitation): On acid agglutination as a method of differentiation of bacteria. 79 (775).

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MEDICAL COLLEGE

NEW YORK CITY

APRIL 16, 1913

AND

THIRD MEETING

PACIFIC COAST BRANCH, SAN FRANCISCO, CALIFORNIA

APRIL 2, 1913

VOLUME X

No. 4

NEW YORK

1913

CONTENTS.

FRANK W. BANCROFT: Heliotropism and galvanotropism in Euglena. 80 (776).

R. A. LAMBERT: The fat content, morphology and length of life of cells growing in diluted blood plasma. 81 (777).

J. F. MCCLENDON: The rate of absorption of water by the skin of the frog, in relation to M. H. Fischer's theory of edema. 82 (778).

J. F. MCCLENDON: The dynamics of a model of cell division. 83 (779).

T. S. GITHENS and S. J. MELTZER: Antitoxic action of sodium iodide on morphin. 84 (780).

E. B. MEIGS: On the nature of the semipermeable membranes which surround the fibres of striated muscle. 85 (781).

H. H. JANEWAY and E. M. EWING: The mechanical factors of excessive artificial respiration and a consideration of their relation to the acapnial theory of shock. 86 (782).

J. L. TODD: A note on the transmission of spirochaetes. 87 (783).

A. J. GOLDFARB: Experimentally fused larvae. 88 (784).

J. ROSENBLOOM and B. A. COHOE: Metabolism studies in a case of myotonia atrophica. 89 (785).

N. B. FOSTER: Sugar from lactic acid in human diabetes. 90 (786).

J. A. ATKINSON and C. B. FITZPATRICK: On some vaccinia blood pressor substances in rabbits. 91 (787).

T. BRAILSFORD ROBERTSON and THEODORE C. BURNETT: Preliminary communication on the part played by cholesterol in determining the incidence of carcinoma. 92 (788).

T. BRAILSFORD ROBERTSON and THEODORE C. BURNETT: The influence of digitonin upon the growth of carcinoma. 93 (789).

THEODORE C. BURNETT: Note on the relation of alexin to thrombin. 94 (790).

HANS ZINSSER: On the nature of the union of alexin with specific precipitates. 95 (791)

J. G. FITZGERALD and GERTRUDE MACKINTOSH: Action of gentian violet on Mucosus capsulatus group. 96 (792).

S. W. YOUNG (by invitation): On the influence of light on the electrical potential of bacterial and other suspensions. 97 (793).

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COLUMBIA UNIVERSITY

NEW YORK CITY,

MAY 21, 1913

VOLUME X

No. 5

NEW YORK

1913

CONTENTS.

E. E Butterfield : The color index and color of the red blood corpuscles. 98 (794).

S. J. Meltzer and J. Auer : Combined action of magnesium and ether ; evidence for a central effect of magnesium. 99 (795).

Peyton Rous : Note on the production of acid by tissues growing in vitro. 100 (796).

C. R Stockard : The location of the optic anlage in Amblystoma and the interpretation of certain eye defects. 101 (797).

D. Wright Wilson : The occurrence of betaine in the muscles of invertebrates. 102 (798).

E. C. Kendall : The chemical constituents of the thyroid gland. 103 (799).

E. C. Kendall : The specific physiological activity of certain constituents of the thyroid gland. 104 (800).

M. S. Fine and V. C. Myers : Further studies on muscle creatine. 105 (801).

J. H. Austin and C. B. Farr : The total non-protein nitrogen of the blood in nephritis and allied conditions. 106 (802).

W. Salant and C. S. Smith : The toxicity of sodium tartrate with special reference to diet and tolerance. 107 (803).

J. R. Murlin and B. Kramer : The influence of pancreatic and duodenal extracts on the glycosuria and the respiratory metabolism of depancreatized dogs. 108 (804).

J. R. Murlin, L. Edelmann and C. W. Giles : The carbon dioxide and oxygen content of the blood after ligation of the abdominal aorta and the inferior vena cava. 109 (805).

J. E. Sweet, E. P. Corson-White and G. M. Saxon : The influence of certain diets upon the growth of experimental tumors. 110 (806).

Charles E. Simon and Martha A. Wood : On the inhibitory action of certain anilin dyes upon bacterial development. 111 (807).

R. M. Pearce : Waxy degeneration of muscle in venom intoxication. 112 (808).

Isaac Ott and J. C. Scott : Note on the effect of animal extracts upon the volume of the thyroid gland. 113 (809).

Sutherland Simpson : The relation of external temperature to hibernation. 114 (810).

Richard Weil : Anaphylaxis in immune animals. 115 (811).

A. J. Carlson : The parathyroids and pregnancy. 116 (812).

A. J. Carlson : The correlation between the physiological state of the thyroid of the mother and of the fetus. 117 (813).

A. J. Carlson : Parathyroid tetany and active immunity. 118 (814).

A. J. Carlson and A. Woelfel : The solubility of various lead salts in the human gastric juice and its bearing on the hygiene of the lead industries. 119 (815).

A. M. Banta and R. A. Gortner : Certain observations on the occurrence of tyrosinase in amphibian egg jell. 120 (816).

A. M. Banta : Experiments on the light and tactile reactions of a cave variety and open water variety of an amphipod species. 121 (817).

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Dates of the next two regular meetings:

April 16, 1913—May 21, 1913.

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